

# Cystic Fibrosis Gene Mutations and Pancreatitis Risk: Relation to Epithelial Ion Transport and Trypsin Inhibitor Gene Mutations

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See editorial on page 1508.

**Background & Aims:** Nonalcoholic chronic pancreatitis is usually idiopathic and often associated with cystic fibrosis gene (*CFTR*) mutations. It is unknown whether pancreatitis risk correlates with having 1 or 2 *CFTR* mutations, abnormal epithelial ion transport, or mutations of other genes. **Methods:** We tested 39 patients with idiopathic chronic pancreatitis (mean age at diagnosis, 33 years) for common mutations of *CFTR* and of genes encoding a trypsin inhibitor (*PSTI*) and trypsinogen (*PRSS1*). To exclude hereditary pancreatitis, we initially relied on family history and subsequently tested for *PRSS1* mutations. Twenty subjects were tested for rare *CFTR* mutations (DNA sequencing) and 11 were tested for extrapancreatic *CFTR* function (clinical and physiologic evaluation). **Results:** Mutations were identified in 24 of 39 subjects. Nine patients had cystic fibrosis-causing mutations, 8 of whom also had mild-variable mutations. Eight others had only mild-variable mutations. Nine subjects had the N34S *PSTI* mutation and 1 had hereditary pancreatitis (R122H, *PRSS1*). Pancreatitis risk was increased approximately 40-fold by having 2 *CFTR* mutations ( $P < 0.0001$ ), 20-fold by having N34S ( $P < 0.0001$ ), and 900-fold by having both ( $P < 0.0001$ ). Subjects with 2 *CFTR* mutations had abnormal nasal epithelial ion transport and clinical findings suggesting residual *CFTR* function between that in cystic fibrosis and in carriers. By contrast, subjects with only *PSTI* mutations had normal *CFTR* function. **Conclusions:** *CFTR*-related pancreatitis risk correlates with having 2 *CFTR* mutations and reduced extrapancreatic *CFTR* function. The N34S *PSTI* mutation increased risk separately. Testing for pancreatitis-associated *CFTR* and *PSTI* genotypes may be useful in nonalcoholic pancreatitis.

sporadic,<sup>2,3</sup> recent studies have associated this condition with mutations in *CFTR*, the gene that causes cystic fibrosis (CF).<sup>4,5</sup>

It is unknown whether pancreatitis risk is mainly associated with having 1 or 2 *CFTR* mutations. This is important because CF carriers (with 1 CF-causing mutation) represent 3%–4% of the general population. Combining the data from several recent studies, 18% of individuals with idiopathic chronic pancreatitis had common CF-causing mutations, whereas only 2% were recognized as compound heterozygotes who had both a milder *CFTR* mutation and a CF-causing mutation.<sup>4–9</sup>

We suspected that these published studies underestimated the frequency of *CFTR* compound heterozygotes in pancreatitis because they did not comprehensively test for rare *CFTR* mutations. Thus, one goal of this study was to perform more detailed genetic testing to determine the prevalence of rare *CFTR* mutations in individuals who would have been classified as CF carriers based on routine genetic testing.

It is also unknown whether other genes modify the risk of *CFTR*-related pancreatitis. Mutations in the pancreatic secretory trypsin inhibitor gene (*PSTI* or *SPINK1*) have recently been associated with childhood pancreatitis.<sup>10,11</sup> Because *PSTI* expression is acinar<sup>12</sup> and *CFTR* is ductular,<sup>13</sup> we anticipated that these genes might independently influence pancreatitis risk. If so, then testing for *PSTI* mutations might identify a subset of CF carriers in whom the risk of pancreatitis is especially high.<sup>12,14</sup>

To better define the link between *CFTR* mutations and pancreatitis risk, we performed detailed testing on a series of patients with idiopathic chronic pancreatitis. The series includes subjects from our prior report<sup>4</sup> plus additional subjects recruited using similar criteria. We

Nonalcoholic chronic pancreatitis commonly leads to recurrent abdominal pain, pancreatic insufficiency, and life-threatening complications.<sup>1</sup> Even though nonalcoholic chronic pancreatitis is usually idiopathic and

Abbreviation used in this paper: CF, cystic fibrosis.

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examined them for rare *CFTR* mutations, for *PSTI* mutations, and for clinical and physiologic evidence of impaired extrapancreatic *CFTR* function. By correlating pancreatitis risk with reduced *CFTR*-mediated ion transport and related clinical features, this analysis has implications for the diagnosis, pathogenesis, and prevention of pancreatitis.

## Materials and Methods

### Study Subjects

Our prior study examined 27 subjects who were identified by reviewing charts of patients referred to Duke University Medical Center from 1991 to 1996, as described.<sup>4</sup> Briefly, each subject was white and had multiple episodes of abdominal pain with objective evidence of pancreatitis; we excluded patients with moderate alcohol use (over 15 drinks/week at any time), hereditary pancreatitis by family history, or CF lung disease.<sup>4</sup> When subsequent genetic testing diagnosed hereditary pancreatitis<sup>15</sup> in 2 of the initial report's subjects (nos. 11 and 15),<sup>4</sup> we recontacted them to further review their family histories and recognized that 1 case (no. 15) should have originally been excluded on this basis; therefore, 26 subjects from the prior report<sup>4</sup> met the original entry criteria and were included in this series. In subsequent chart reviews, we focused on younger patients (diagnosed by age 50 years) and we screened potential new subjects for *PRSS1* mutations before entry to avoid diluting the series of idiopathic cases with individuals who have hereditary pancreatitis. Using these modified entry criteria for pancreatitis patients seen at this medical center, 8 additional subjects were enrolled and 3 candidates were excluded because they had the R122H *PRSS1* mutation; the family histories were negative for the 3 excluded candidates. In addition, we enrolled 3 patients who were referred from outside centers who met these entry criteria.

Two additional patients were tested who were referred from outside centers as known CF carriers with unexplained pancreatitis, nondiagnostic sweat tests, and normal chest radiographs. They were tested for rare *CFTR* mutations and for mutations of other genes; also, 1 of these subjects underwent clinical and physiologic evaluation studies (see below). They were excluded from the series for most prevalence ratio calculations because they were known to be CF carriers before referral. Thus, the full series contained 39 subjects, but most prevalence calculations were based on data for only 37 subjects. Table 1 summarizes how subgroups of subjects were recruited and tested.

### Molecular Analyses

All subjects were screened for 17 *CFTR* mutations (including 5T) as described<sup>4</sup> and were tested for D1152H by restriction enzyme digestion. In 20 subjects, including all 13 subjects with only one mutation detected by screening, we sequenced all *CFTR* exons and flanking regions (ABI310 Genetic Analyzer; Applied Biosystems, Foster City, CA). The sequenced group included 4 subjects with normal *CFTR* genotypes (by screening) who were selected based on their willingness to undergo physiologic testing (see below). Sequencing was not done in others with normal *CFTR* genotypes (by screening) because of cost.

We defined severe alleles ( $CF^{sev}$ ) as those causing CF by current consensus criteria<sup>16</sup>; they cause classic CF in homozygotes ( $CF^{sev}/CF^{sev}$ ) and in compound heterozygotes who also have  $\Delta F508$  ( $\Delta F508/CF^{sev}$ ). Mild-variable alleles ( $CF^{m-v}$ ) cause variable phenotypes (e.g., congenital bilateral absence of the vas deferens) in compound heterozygotes with  $CF^{sev}$  alleles ( $CF^{sev}/CF^{m-v}$ ).  $CF^{m-v}$  includes mutations that sometimes cause classic CF (e.g., D1152H) and mutations that predispose to congenital bilateral absence of the vas deferens even though most compound heterozygotes are asymptomatic (e.g., 5T).

*PSTI* exons and flanking sequences were screened by denaturing high-performance liquid chromatography.<sup>17,18</sup> N34S

**Table 1.** Subgroups of Subjects Based on Recruitment and Testing

Subgroups based on recruitment	
39	Total number of subjects in study.
26	Number of subjects recruited for the initial study. <sup>4</sup> For these, hereditary pancreatitis was excluded based on family history.
11	Number of subjects recruited using similar inclusion criteria after the initial study. For these, hereditary pancreatitis was excluded based on <i>PRSS1</i> genetic testing.
2	Number of subjects who were known to be CF carriers before referral. These subjects were excluded from most prevalence ratio calculations.
Subgroups based on testing	
39	Number of subjects screened for common <i>CFTR</i> , <i>PSTI</i> , and <i>PRSS1</i> mutations.
20	Number of subjects who had comprehensive DNA sequencing of <i>CFTR</i> gene.
8	Subjects who had 1 CF-causing mutation found by screening.
8	Subjects who had only mild-variable ( $CF^{m-v}$ ) mutations identified by screening.
4	Subjects who had 2 apparently normal <i>CFTR</i> alleles based on screening.
11	Number of subjects who had physiologic and clinical testing (Table 4).
6	<i>CFTR</i> compound heterozygotes.
1	CF carrier.
4	Subjects with normal <i>CFTR</i> genotypes.

heterozygotes and homozygotes were confirmed by sequencing exon 3 and restriction enzyme digestion (HpyCH4 III); heterozygotes were further tested by sequencing the *PSTI* promoter, exons, and flanking sequences. To estimate the general prevalence of N34S, white patients with hemochromatosis or fragile X were screened by denaturing high-performance liquid chromatography and restriction enzyme digestion; N34S occurred in 4 of 426 control alleles (0.9%).

For *PRSS1*, DNA was tested for R122H ("R117H") by restriction enzyme digestion<sup>15</sup> and for A16V ("A8V") and N29I ("N21I") by sequencing exon 2.<sup>19,20</sup>

### Physiologic and Clinical Studies

Eleven subjects were selected for physiologic and clinical evaluation based on their willingness to participate. Nasal bioelectric responses were recorded to measure baseline potential differences and CFTR-mediated ion transport.<sup>21</sup> Subjects were clinically assessed for respiratory and male reproductive findings. Laboratory studies included sweat testing, chest radiographs, spirometry, and sputum culture. Subjects provided

separate informed consent for genetic and physiologic/clinical testing.

### Data Analysis

Groups were compared using the Fisher exact or unpaired *t* tests. The exact test was used for comparisons yielding prevalence ratios below 500 and the binomial distribution was used for comparisons involving very rare events (with prevalence ratios over 500). Means ( $\pm$  SEM) and 2-tailed *P* values are presented.

## Results

### CFTR Mutations in Individual Subjects

Twenty-six *CFTR* mutations were detected in 17 subjects (Table 2). There were 21 common mutations; 9 were severe ( $CF^{sev}$ :  $\Delta F508$ , N1303K, 621+1G>T) and 12 were mild-variable ( $CF^{m-v}$ : 5T, R117H). Among the 9 subjects with severe mutations (nominal CF carriers), 3

**Table 2.** Abnormal *CFTR*, *PSTI*, and *PRSS1* Alleles Detected in 39 Patients With Chronic Pancreatitis

No.	Age at Dx	Sex	Abnormal <i>CFTR</i> genotypes	<i>CFTR</i> polymorphisms and complex alleles <sup>a</sup>	<i>PSTI</i>	<i>PRSS1</i>
1	45	M	$\Delta F508/R117H-7T$	(Screened only)	N34S/-	-/-
2	32	F	$\Delta F508/5T$	11TG-5T-V470; I1027T	-/-	-/-
3	48	F	$\Delta F508/5T$	12TG-5T-V470	-/-	-/-
4 <sup>b</sup>	8	M	$\Delta F508/D1152H$	D1152H-V470	-/-	-/-
5	15	F	$\Delta F508/D1152H$	D1152H-V470	-/-	-/-
6	26	F	$\Delta F508/P574H$	None	-/-	-/-
7	40	F	$\Delta F508/3120G>A$	None	-/-	-/-
8 <sup>b</sup>	16	M	621+1G>T/G1069R	None	-/-	-/-
9	7	F	5T/5T	13TG-5T/11TG-5T	N34S/-	-/-
10	43	M	N1303K/-	None	-/-	-/-
11	32	F	R117H-7T/-	4005+117T>G	-/-	-/-
12	33	M	5T/-	1716G>A/12TG-5T-V470	-/-	-/-
13	51	M	5T/-	11TG-5T	-/-	-/-
14	29	F	5T/-	None	-/-	-/-
15	12	F	5T/-	None	-/-	-/-
16	30	F	5T/-	1248+15T>A/11TG-5T	-/-	-/-
17	14	M	5T/-	12TG-5T-V470	N34S/-	-/-
18	12	F	-/-	None	N34S/N34S	-/-
19	46	F	-/-	R75Q	N34S/-	-/-
20	44	F	-/-	None	N34S/-	-/-
21	21	F	-/-	(Screened only)	N34S/-	-/-
22	47	F	-/-	(Screened only)	N34S/-	-/-
23	35	M	-/-	(Screened only)	N34S/-	-/-
24	16	F	-/-	R75Q	-/-	R122H/-

NOTE. Thirty-nine ICP patients were screened for common *CFTR*, *PSTI*, and *PRSS1* mutations. The *CFTR* mutation panel included  $\Delta F508$ , R117H, R334W, R347P, A455E, I507, G542X, S549N, G551D, R553X, R560T, D1152H, W1282X, N1303K, 621+1G>T, 1717-1G>A, 3849+10kb C>T and the 5T allele in intron 8. Most *CFTR* mutation screening data was previously reported for 16 subjects: nos. 1-3, 5, 7, 10-12, 14, 15, 18, 19, and 21-24.<sup>4</sup> Twenty subjects were tested for rare *CFTR* mutations by sequencing all exons and flanking regions (nos. 2-20 and 24). Eight subjects were tested for rare *PSTI* mutations by sequencing all exons, flanking regions, and the promoter (nos. 1, 9, 17, and 19-23).

Dx, diagnosis.

<sup>a</sup>I1027T, R75Q, and 1716G>A are noted because they may be associated with congenital bilateral absence of the vas deferens.<sup>22,28</sup> 1248+15T>A is novel (significance unknown). Complex alleles cause greater loss of function than 5T alone (e.g., 13TG-5T, 12TG-5T, and 5T-V470).<sup>28</sup> Mutations linked to V470 are noted because V470 reduces CFTR function by 40%.<sup>28</sup> Six subjects were heterozygous for V470 with undetermined linkage (nos. 10, 13-15, 19, and 24). All 20 sequenced subjects were tested for the above polymorphisms plus 2694T>G (T854T), 4521G>A (Q1463Q), 405+46G>T, 875+40A>G, IVS6a(GATT)6-7, and 1001+11C>T.

<sup>b</sup>Before referral, subjects 4 and 8 were known to have the  $\Delta F508$  or 621+1G>T mutations with normal or borderline sweat testing, respectively.

had common milder *CFTR* mutations (nos. 1–3), 5 had rare mutations that were detected by DNA sequencing (nos. 4–8), and 1 had a normal second *CFTR* allele based on DNA sequencing (no. 10). Each of the rare mutations detected in subjects 4–8 is mild-variable: D1152H, P574H, G1069R, and 3120G>A.<sup>22–25</sup> No other rare mutations were found in the remaining 11 subjects who were tested by DNA sequencing.

### Compound Heterozygotes

Nine subjects were compound heterozygotes. Eight had CF<sup>sev</sup>/CF<sup>m-v</sup> genotypes (nos. 1–8) and 1 was CF<sup>m-v</sup>/CF<sup>m-v</sup> (no. 9). None of the subjects had 2 severe mutations, as anticipated, because individuals with CF were excluded. Only 1 compound heterozygote's genotype was detectable by routine genetic testing (no. 1).

### Novel Mutations, Polymorphisms, and Complex Alleles

One subject had a new mutation (no. 16; 1248+15T>A; significance unknown). No polymorphism was especially frequent in pancreatitis; 3 of the detected polymorphisms may predispose to male infer-

tility and might therefore be associated with partial loss of *CFTR* function (I1027T, 1716G>A, R75Q).<sup>22,26,27</sup> The 5T mutation often occurred in complex alleles (in *cis*) that cause greater loss of function than 5T alone: TG13-5T (no. 9), TG12-5T-V470 (nos. 3, 12, and 17), and 5T-V470 (no. 2).<sup>28–31</sup>

### CFTR Allele and Genotype Prevalences

Table 3 compares the observed versus expected frequencies of *CFTR* alleles and genotypes in the series. Pancreatitis is associated with severe mutations (5:1, observed:expected ratio). However, the more striking association is with CF<sup>sev</sup>/CF<sup>m-v</sup> genotypes (80:1).

Among the 9 subjects with severe mutations, 8 had mild-variable mutations (16:1); only 1 CF carrier had a normal allele (no. 10). Compound heterozygotes (nos. 1–9) accounted for most detected severe (8/9) and mild-variable (10/17) mutations. After excluding compound heterozygotes, pancreatitis was no longer significantly associated with severe (1/29) or mild-variable (7/29) mutations. There also was no association with 5T after excluding subjects with complex alleles containing 5T (4/33).

**Table 3.** Prevalence Ratios for *CFTR* Mutations and Genotypes in Idiopathic Chronic Pancreatitis

Genotypes or alleles	Frequencies		Ratio (observed:expected)
	Expected <sup>a</sup>	Observed <sup>b</sup>	
Abnormal <i>CFTR</i> alleles			
CF <sup>sev</sup> (severe)	1/57	7/74	5.3 [2.5–11.7], <i>P</i> = 0.0006
CF <sup>m-v</sup> (mild-variable)	1/18	17/74	4.1 [2.6–6.5], <i>P</i> < 0.0001
5T (most common CF <sup>m-v</sup> )	1/20	10/74	2.6 [1.5–5.0], <i>P</i> < 0.005
Abnormal <i>CFTR</i> genotypes			
Compound heterozygotes	1/204	7/37	39 [14–105], <i>P</i> < 0.0001
CF <sup>sev</sup> /CF <sup>m-v</sup>	1/509	6/37	83 [21–318], <i>P</i> < 0.0001
Abnormal <i>PSTI</i> alleles			
N34S	11/1764	10/74	22 [10–49], <i>P</i> < 0.0001
In subjects Dx'd by age 21	"	5/18	45 [17–115], <i>P</i> < 0.0001
In subjects Dx'd after age 21	"	5/56	14 [5–40], <i>P</i> < 0.0001
<i>CFTR</i> compound heterozygote + N34S	1/16400	2/37	900, <i>P</i> < 0.0001
Subset analyses			
CF <sup>m-v</sup> , if CF <sup>sev</sup> present	1/18	8/9	16 [13–20], <i>P</i> < 0.0001
N34S, if <i>CFTR</i> compound heterozygote	11/1764	2/18	18 [4–75], <i>P</i> = 0.007

NOTE. Subject numbers and genotypes are listed in Table 2. Abnormal *CFTR* alleles cause either severe (CF<sup>sev</sup>) or mild-variable loss of function (CF<sup>m-v</sup>). Observed:expected ratios and 95% confidence intervals (in brackets) were calculated by the Fisher exact test when possible. For ratios over 500, *P* values were calculated using the binomial distribution.

Dx'd, diagnosed.

<sup>a</sup>The expected frequency of mutations was estimated assuming that the frequency of CF in U.S. Whites is 1/3200,<sup>51</sup> that the total allele frequency for CF<sup>sev</sup> mutations is 1/3200<sup>0.5</sup>, and that the allele frequencies for 5T and R117H are 5%<sup>23,24,52</sup> and 0.2%,<sup>53,54</sup> respectively. Allele prevalence data is unavailable for other CF<sup>m-v</sup> alleles; in congenital bilateral absence of the vas deferens, their combined prevalence is less than for R117H<sup>24,26,27</sup> and we assumed that their combined prevalence in U.S. Whites is 0.4%. For the N34S *PSTI* mutation, the expected allele frequency was calculated by combining data from 3 prior studies (7/1338 alleles, total)<sup>10,11,32</sup> with data for control subjects in this study (4/426 alleles). To estimate expected frequencies containing multiple alleles, we assumed that each occurred independently (e.g., CF<sup>sev</sup>/CF<sup>m-v</sup> = (2)(1/18)(1/56.6) = 1/509).

<sup>b</sup>A total of 26 abnormal *CFTR* alleles were detected among 78 tested alleles in 39 patients. However, because 2 subjects were known to have CF<sup>sev</sup> mutations before enrollment (nos. 4 and 8), their data was excluded from most calculations to avoid ascertainment bias. These 2 subjects were only included in the subset analyses at the bottom of the table.

### PSTI Mutations

The N34S *PSTI* mutation occurred in 10 of 78 alleles from study subjects (21:1;  $P < 0.0001$ ). One subject was N34S homozygous, and 8 were heterozygotes with 1 verified normal *PSTI* allele. Thus, N34S carrier status is sufficient to increase pancreatitis risk. Other potential *PSTI* mutations (M1T, L14P, D50E, P55S, IVS2-23A>T, and IVS3+2T>C)<sup>10,11,32</sup> were absent.

Two of 9 *CFTR* compound heterozygotes with pancreatitis had N34S (18:1;  $P < 0.01$ ). Assuming independent inheritance of *CFTR* and *PSTI* genotypes, these 2 examples suggest that pancreatitis risk is increased approximately 900-fold in N34S carriers who are *CFTR* compound heterozygotes.

### PRSS1 Mutations

The R122H *PRSS1* mutation occurred in 1 subject who was enrolled when we were excluding hereditary pancreatitis (pre-entry) based only on family history data (no. 24). Anamnestic data for this subject indicated that she had no relatives with pancreatitis. For subjects recruited after 1996, hereditary pancreatitis was excluded before entry by *PRSS1* testing. Accordingly, R122H and other known *PRSS1* mutations were absent in all patients in the series other than no. 24.

### Clinical and Physiologic Features

The mean age at diagnosis was  $33.2 \pm 2.5$  years ( $n = 39$ ), and 11 subjects were diagnosed by age 21 years.

Subjects 2, 3, 4, and 8 each had 1 relative with pancreatitis. The affected sister of subject 8 had the genotype 621+1G>T / G1069R; DNA was not available for the other 3 affected relatives. Subjects with any mutation (no. 1–24) were younger than others at diagnosis ( $29.3 \pm 2.9$  vs.  $39.5 \pm 4.2$ ;  $P < 0.05$ ). Pancreatogram findings were equally severe in subjects with or without mutations.

Table 4 compares physiologic data for the tested subjects who had 0 vs. 2 *CFTR* mutations. Five of these subjects had *PSTI* or *PRSS1* mutations; the physiologic parameters were unaffected by these mutations. (Subjects were selected for physiologic testing before testing for *PSTI* mutations was possible.) Baseline nasal potential difference (PD) values, which reflect basal  $\text{Na}^+$  transport, were normal and did not correlate with the number of *CFTR* mutations.

### CFTR-Mediated Chloride Transport

$\Delta$ PD values (in mV) correlated with *CFTR* genotypes in subjects with 0 vs. 2 mutations (Figure 1). When compared with historical data,<sup>21</sup> subjects with no mutations resembled normal controls. In compound heterozygotes, *CFTR*-mediated chloride transport was lower than in normal controls ( $P < 0.0001$ ) or in subjects without mutations ( $P < 0.01$ ), but higher than in individuals with CF ( $P < 0.002$ ).<sup>21</sup>

### Sweat Testing

Sweat chloride values were normal in subjects with normal *CFTR* genotypes who had *PRSS1* or *PSTI*

**Table 4.** Clinical and Physiologic Data for 11 Patients With Pancreatitis

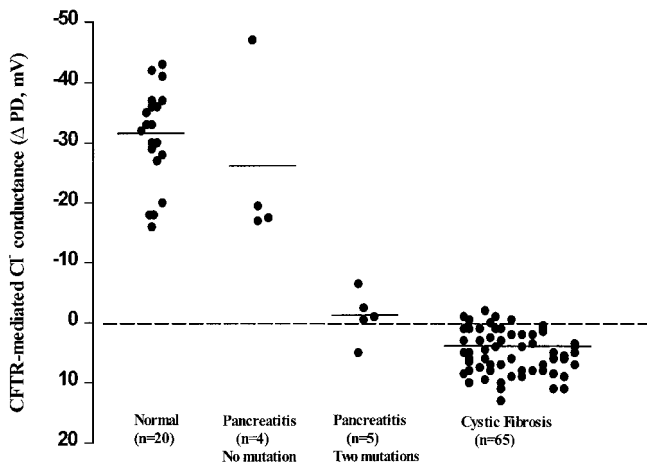
No.	Age, sex	<i>CFTR</i> genotype	Sweat $\text{Cl}^-$ (mmol/L)	FEV <sub>1</sub> (% predicted)	Baseline PD (mV)	$\Delta$ PD (mV)	<i>PSTI</i> or <i>PRSS1</i> mutations, sputum cultures, male fertility, etc.
1	47, M	$\Delta$ F508/R117H	29	86	-24	+5.0	N34S/–, infertile, <sup>a</sup> smooth <i>P. aeruginosa</i>
2	34, F	$\Delta$ F508/5T	59	106	-15	+1.5	
3	53, F	$\Delta$ F508/5T	62	78	-10	-1.0	FEV <sub>1</sub> 58% before subject stopped smoking
4	18, M	$\Delta$ F508/D1152H	62	115	-38	-6.5	Palpable vas deferens
5	21, F	$\Delta$ F508/D1152H	32	94	-23	-2.5	
6	28, F	$\Delta$ F508/P574H	81	109	-25	ND	<i>S. aureus</i> , <i>S. maltophilia</i> ; sinus surgery
10	47, M	N1303K/–	41	106	-20	-20	Palpable vas deferens
18	20, F	–/–	24	119	-27	-18	N34S/N34S
19	50, F	–/–	36 <sup>b</sup>	31	-13	-17	N34S/–, 30 pack/year smoker
20	46, F	–/–	16	97	-10	-47	N34S/–
24	18, F	–/–	14	86	-16	-19.5	R122H cationic trypsinogen mutation
Mean values ( $\pm$ SEM) for groups based on <i>CFTR</i> genotypes							
1–6		mutant/mutant	$54 \pm 8^c$	$98 \pm 6$	$-23 \pm 4$	$-0.7 \pm 1.9^c$	
18–20 and 24		–/–	$23 \pm 5$	$83 \pm 19$	$-17 \pm 2$	$-25.4 \pm 7.3$	

NOTE. Subject numbers and genotyping data are from Table 2. This table shows age at testing (not age at diagnosis). Each subject's chest radiograph was normal except for basilar linear atelectasis in no. 1 and hyperinflation in subjects 13 and 19. Some data was previously reported for subjects 1–3.<sup>4</sup> Subject 3 was retested after she stopped smoking. Other subjects were nonsmokers except as noted.

<sup>a</sup>Subject 1 was infertile because of congenital bilateral absence of the vas deferens. Subjects 4 and 10 had a palpable vas deferens and subject 10 had offspring.

<sup>b</sup>This value is based on 1 measurement; other values are means of at least 2 sweat tests.

<sup>c</sup>Subjects with 2 *CFTR* mutations were significantly different from subjects with no mutations based on sweat  $\text{Cl}^-$  and  $\Delta$ PD values ( $P < 0.02$  for each comparison).



**Figure 1.** CFTR function in the nasal epithelium in genotyped subjects with pancreatitis. CFTR-mediated  $\text{Cl}^-$  conductance ( $\Delta\text{PD}$ ) was measured in subjects with pancreatitis who had 0 vs. 2 *CFTR* mutations, and compared with published values for normal controls and for individuals with classic cystic fibrosis.<sup>20</sup> Subjects with CFTR-related pancreatitis (i.e., those with 2 mutations) differed significantly from subjects without *CFTR* mutations, from normal controls, and from individuals with CF based on their  $\Delta\text{PD}$  values ( $P < 0.01$  for each comparison). This indicates that these subjects had less CFTR-mediated ion transport than normal but that this loss of function was less severe than in cystic fibrosis. One study subject with a CF-causing mutation had a normal second *CFTR* allele based on DNA sequencing; his  $\Delta\text{PD}$  value of  $-20$  mV (not shown) was normal, as anticipated based on published data for obligate CF carriers.<sup>32</sup>

mutations. By contrast, subjects with 2 *CFTR* mutations had higher mean sweat chloride values ( $54 \pm 8$ ,  $P < 0.02$ ). Even though these subjects each had at least 2 normal or borderline sweat chloride values in the past, 3 of them had values above 60 mmol/L when last tested (Table 4).

### Respiratory Findings

No subject had CF lung disease (Table 4). Nonetheless, 2 compound heterozygotes produced sputum containing CF-associated bacteria (nos. 1 and 6); in both, spirometry was normal and bronchiectasis was absent. One compound heterozygote (no. 6) had severe recurrent sinusitis requiring a Caldwell-Luc procedure; others (nos. 1 and 3) had milder sinusitis.

### Fertility in Males

One compound heterozygote had congenital bilateral absence of the vas deferens (no. 1); another had a palpable vas bilaterally (no. 4).

### Features of the CF Carrier

One subject in the series was a CF carrier who had 1 normal *CFTR* allele based on DNA sequencing (no. 10). He had normal baseline and  $\Delta\text{PD}$  values (Table 4), as anticipated based on published data for obligate CF

carriers.<sup>33</sup> He had a palpable vas deferens bilaterally, has fathered children, and had no respiratory findings suggesting reduced CFTR function.

## Discussion

This study shows that idiopathic chronic pancreatitis is often associated with having 2 *CFTR* mutations or 1 *PSTI* mutation. These genotypes occurred in many cases diagnosed by age 50 years (16/32 after excluding hereditary pancreatitis) and in most diagnosed by 21 years old (7/10). Pancreatitis risk was highest in those with 2 *CFTR* mutations plus N34S.

For *CFTR*, the greatest risk of pancreatitis is associated with compound heterozygote genotypes containing 1 severe mutation plus 1 mild-variable mutation. The mild-variable mutations in these genotypes account for the residual CFTR function (5%–25%) that protects these individuals from CF lung disease<sup>24</sup> and that may also be required for pancreatitis to occur.<sup>34</sup> Because mild-variable mutations are generally excluded from mutation panels used for CF testing, routine genetic testing would incorrectly classify most compound heterozygote pancreatitis patients as carriers; this explains why we found a higher frequency of *CFTR* compound heterozygotes than others who tested only for common mutations.<sup>5–9</sup> Thus, even though pancreatitis risk is increased in individuals identified as carriers by routine genetic testing, more complete testing of a series of such nominal carriers with pancreatitis showed that most were compound heterozygotes who had initially undetected mutations. This is the main finding of the present study; it suggests autosomal recessive inheritance for most CFTR-related pancreatitis risk and implicates reduced CFTR function in pathogenesis.

A primary goal of this study was to thoroughly test a series of subjects with pancreatitis who have common CF-causing mutations to determine whether their second *CFTR* allele contains an uncommon mutation that was initially undetected. We tested for these uncommon mutations by DNA sequencing because it is more sensitive than the screening methods previously used to study pancreatitis<sup>4–9</sup> and other nonpulmonary CFTR-related diseases.<sup>22–26,35–37</sup> Because some subjects in this study (a minority) were not tested by DNA sequencing, it is possible that our data underestimated the prevalence of genotypes containing only mutations that were absent in the mutation panel used for screening (e.g.,  $\text{CF}^{\text{m-v}}/\text{CF}^{\text{m-v}}$  genotypes).

This study found mild-variable *CFTR* mutations in most pancreatitis patients with abnormal genotypes. The predominant mild-variable mutation in pancreatitis was

5T. This mutation causes inefficient splicing of exon 10 leading to production of nonfunctional copies of CFTR.<sup>38</sup> 5T occurred in 13% of pancreatitis alleles vs. 5% in the general population and vs. 12%–20% in congenital bilateral absence of the vas deferens.<sup>22,24,26</sup> Our data contrasts with prior studies reporting no association of 5T with pancreatitis.<sup>5–7,9</sup> The higher 5T prevalence in our study may partly result from including subjects with juvenile-onset disease and excluding individuals reporting moderate alcohol use. In any case, our data suggests that 5T-related pancreatitis risk is largely caused by 5T occurring either in complex alleles<sup>28–31</sup> or in *CFTR* compound heterozygote genotypes. This study's physiologic findings also correlate CFTR loss of function with pancreatitis risk. CFTR-mediated chloride transport in the nasal epithelium ( $\Delta$ PD) was abnormal in compound heterozygotes but normal in subjects without *CFTR* mutations. Nonetheless, compound heterozygotes differed from CF patients in that their  $\Delta$ PD values were less abnormal and their baseline PD values were normal; both findings reflect CFTR loss of function between that in CF (>95%) and in carriers (50%). By contrast, the 1 CF carrier in the series (no. 10) had a normal  $\Delta$ PD value, as is typical of obligate CF carriers.<sup>33</sup> Because the prevalence of CF carriers was not increased in the series, this individual may have been ascertained by chance, alone.

Finally, the link between pancreatitis risk and CFTR loss of function is supported by extrapancreatic clinical findings observed in compound heterozygotes. These are reminiscent of findings associated with congenital bilateral absence of the vas deferens<sup>23,25,26,35,36</sup> and include abnormal sweat testing, bronchitis with sputum containing CF-typical pathogens, and sinusitis. The absence of CF lung disease was anticipated because no subject had 2 severe mutations<sup>24</sup>; the variable expression of vas deferens disease was also anticipated based on the incomplete penetrance of infertility in males with CF<sup>sev</sup>/CF<sup>m-v</sup> genotypes (approximately 20%).<sup>39</sup>

There are many parallels between CFTR-related pancreatitis and congenital bilateral absence of the vas deferens.<sup>40</sup> Both diseases are usually sporadic, delaying recognition of their heritability. Both were initially associated with CF carrier status; however, further study revealed their closer association with compound heterozygosity and with mild-variable mutations initially classified as benign polymorphisms (e.g., 5T). As in congenital bilateral absence of the vas deferens,<sup>39</sup> there seems to be incomplete penetrance for pancreatitis in *CFTR* compound heterozygotes, implicating gene-gene or gene-environment interactions in pathogenesis. In other words, since most *CFTR* compound heterozygotes do not develop

pancreatitis, other genetic or environmental factors must also be important in determining this phenotype.

More generally, the relationship between CF carrier status and pancreatitis suggests a novel paradigm for genetic predisposition in which mild mutations of a recessive Mendelian disease gene (*CFTR*) increase the risk of a seemingly unrelated disease (pancreatitis).<sup>40</sup> Based on this example, we speculate that mild or atypical mutations of other Mendelian disease genes may play similar roles in other complex diseases.

For gastroenterologists, there also are pertinent parallels between CFTR-related pancreatitis and some forms of cholestasis with respect to genetic predisposition: The liver resembles the pancreas in that CFTR occurs mainly in the apical membranes of duct cells where it normally promotes bicarbonate and fluid secretion into the duct lumen.<sup>41–43</sup> This suggests that reduced CFTR function might predispose to hepatobiliary disease, and this suggestion is supported by recent data associating reduced nasal CFTR-mediated chloride transport with sclerosing cholangitis.<sup>44</sup>

This study also examined how *PSTI* mutations influence pancreatitis risk. It was already known that having the N34S *PSTI* mutation predisposes to childhood pancreatitis; even though most children with N34S do not develop pancreatitis, the risk of pancreatitis is increased in those who have this mutation.<sup>10,11</sup> Our findings show that this association also applies to adults with idiopathic chronic pancreatitis.

We also found that N34S carrier status and *CFTR* compound heterozygosity had additive effects on pancreatitis risk. Because subjects with only N34S (nos. 18–20) had normal CFTR-mediated ion transport and no clinical evidence of reduced extrapancreatic CFTR function, this study's genetic, physiologic, and clinical findings indicate that *PSTI* and *CFTR* mutations act separately to increase pancreatitis risk. One implication of these findings is that intrapancreatic protease activation may contribute to the pathogenesis of CFTR-related pancreatitis. A related implication is that N34S carrier status may help explain the occasional occurrence of pancreatitis in individuals with CF or congenital bilateral absence of the vas deferens.

The effect of *PSTI* and *CFTR* mutations on pancreatitis risk has important implications for pathogenesis and prevention. Because *PSTI* and *CFTR* occur in acinar and ductal cells, respectively, this effect identifies these sites as distinct targets for interventions aiming to reduce pancreatitis risk. Specifically, susceptible individuals may benefit from promoting ductal bicarbonate secre-

tion<sup>41</sup> and from reducing intra-acinar trypsinogen activation.<sup>11</sup>

This study also has implications for clinical practice. For *PRSS1*, we identified one apparently sporadic case of hereditary pancreatitis in the series and 3 additional sporadic cases were excluded among younger candidates considered for this study. Current clinical practice relies on family history data to identify potential cases of hereditary pancreatitis,<sup>19,45,46</sup> but the occurrence of such sporadic cases implies that a negative family is not sufficient to exclude the diagnosis of hereditary pancreatitis.

The frequency of *CFTR*, *PSTI*, and *PRSS1* mutations in chronic pancreatitis suggests that some patients with this condition may benefit from genetic testing. Because initial reports have not associated alcoholic pancreatitis with these mutations,<sup>8,32,47,48</sup> genetic testing may help distinguish between alcohol-related and idiopathic cases; detection of mutations may improve medical care and patient satisfaction in cases where physicians might otherwise suspect undisclosed alcoholism. Moreover, when causative *PRSS1* mutations are detected, the correct diagnosis is hereditary pancreatitis, a disease that differs from idiopathic pancreatitis because it leads to pancreatic adenocarcinoma with a cumulative risk approaching 40%.<sup>49</sup> Surveillance may permit early cancer detection in these individuals.

For *CFTR*, there is currently no consensus about the role of genetic testing in idiopathic chronic pancreatitis.<sup>50</sup> Even though current treatment for chronic pancreatitis is the same regardless of whether *CFTR* mutations are found, some patients will wish to be tested so they can better understand their disease. Others will wish to know if they are carriers for CF before having children.

When *CFTR* genetic testing is done in pancreatitis patients, the results will require cautious interpretation. Rare pancreatitis patients will have 2 detected *CFTR* mutations. This group will include patients with unrecognized classic CF in whom pancreatitis precedes overt lung disease; most will have diagnostic sweat tests and may benefit from specialized pulmonary care. More often, genetic testing will detect only 1 mutation. In these cases, the presence of associated findings (borderline sweat testing, male infertility, abnormal nasal bioelectric responses, etc.) should suggest *CFTR* compound heterozygosity. In these cases, compound heterozygosity might be confirmed by 5T testing; however, most such cases will have mild-variable mutations that are undetectable using widely available *CFTR* genetic testing methods.

In summary, most patients with idiopathic chronic pancreatitis had mutations of at least 1 tested gene. Occasionally, pancreatitis occurred as a sporadic condition caused by *PRSS1* mutations; more often, susceptibility to pancreatitis resulted from *CFTR* compound heterozygosity and *PSTI* carrier status. Genetic, clinical, and physiologic data indicate that *CFTR* and *PSTI* mutations influence pancreatitis risk through independent mechanisms. Genetic testing may be useful in selected patients with chronic pancreatitis.

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