

RELATION BETWEEN MUTATIONS OF THE CYSTIC FIBROSIS GENE AND IDIOPATHIC PANCREATITIS

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ABSTRACT

Background It is unknown whether genetic factors predispose patients to idiopathic pancreatitis. In patients with cystic fibrosis, mutations of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene typically cause pulmonary and pancreatic insufficiency while rarely causing pancreatitis. We examined whether idiopathic pancreatitis is associated with *CFTR* mutations in persons who do not have lung disease of cystic fibrosis.

Methods We studied 27 patients (mean age at diagnosis, 36 years), 22 of whom were female, who had been referred for an evaluation of idiopathic pancreatitis. DNA was tested for 17 *CFTR* mutations and for the 5T allele in intron 8 of the *CFTR* gene. The 5T allele reduces the level of functional CFTR and is associated with an inherited form of infertility in males. Patients with two abnormal *CFTR* alleles were further evaluated for unrecognized cystic fibrosis-related lung disease, and both base-line and CFTR-mediated ion transport were measured in the nasal mucosa.

Results Ten patients with idiopathic chronic pancreatitis (37 percent) had at least one abnormal *CFTR* allele. Eight *CFTR* mutations were detected (prevalence ratio, 11:1; 95 percent confidence interval, 5 to 23; $P < 0.001$). In three patients both alleles were affected (prevalence ratio, 80:1; 95 percent confidence interval, 17 to 379; $P < 0.001$). These three patients did not have lung disease typical of cystic fibrosis on the basis of sweat testing, spirometry, or base-line nasal potential-difference measurements. Nonetheless, each had abnormal nasal cyclic AMP-mediated chloride transport.

Conclusions In a group of patients referred for evaluation of idiopathic pancreatitis, there was a strong association between mutations in the *CFTR* gene and pancreatitis. The abnormal *CFTR* genotypes in these patients with pancreatitis resemble those associated with male infertility. (N Engl J Med 1998;339:653-8.)

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CHRONIC pancreatitis is a potentially life-threatening disease¹; the most common types are alcohol related and idiopathic. Susceptibility to pancreatitis varies widely.² Even though pancreatitis rarely results from recognized genetic defects,^{3,4} it is unknown whether hereditary factors increase the likelihood of the two predominant forms.

The most common inherited disease of the exocrine pancreas is cystic fibrosis.⁵⁻⁸ In cystic fibrosis,

mutations of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene lead to dysfunction of the lung, sweat glands, vas deferens, and pancreas. Lung disease accounts for most of the complications and deaths related to cystic fibrosis. In persons with normal lung function, *CFTR* mutations cause one type of male infertility, congenital (bilateral) absence of the vas deferens.⁹⁻¹²

The association of abnormal *CFTR* genotypes with congenital absence of the vas deferens led us to consider whether the gene has a role in idiopathic chronic pancreatitis. Even though the pancreatic disease in patients with cystic fibrosis does not usually resemble pancreatitis clinically, two factors suggested that *CFTR* has a role. First, both conditions are often associated with abnormal sweat electrolyte values.^{13,14} Second, in both conditions, the earliest pathological finding is pancreatic ductal obstruction due to inspissated secretions,^{15,16} and the ducts are normally the predominant site of CFTR protein in the pancreas.¹⁷ Because pancreatitis occasionally occurs in patients with cystic fibrosis^{5,18} and because *CFTR* mutations are common, we examined whether abnormal *CFTR* genotypes are predisposing factors for idiopathic pancreatitis.

METHODS**Selection of Patients**

The records of all white patients who were referred to Duke University Medical Center in North Carolina from 1991 to 1996 for an evaluation of chronic pancreatitis were reviewed. Among 32 patients with idiopathic pancreatitis, 27 provided written informed consent and participated in the study. The study protocol was approved by the medical center's institutional review board. Each patient had had at least two episodes of pancreatitis at least six months apart. Each episode met at least two of the three criteria: abdominal pain typical of pancreatitis, an elevation of serum amylase or lipase (more than three times the upper limit of the normal range), and evidence of pancreatitis on abdominal computed tomography. Exclusion criteria included the ingestion of more than 15 alcoholic drinks per week at any time; the presence of hyperlipidemia, pancreatic cancer, pancreas divisum, or dysfunction of the sphincter of Oddi; and the onset of pancreatitis after the age of 65 years. Patients were also excluded if their pancreatitis was related to trauma, gallstones, drugs, or autosomal dominant pancreatitis. Twenty-two of the patients were female

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(81 percent); this preponderance may have resulted from the exclusion of many male patients because of alcohol use. The mean age at the diagnosis of pancreatitis was 36 years (range, 12 to 65). Pancreatograms were assessed for the severity of chronic pancreatitis according to published criteria by a reviewer who was unaware of the patients' histories (Table 1).¹⁹

DNA Studies

We extracted DNA from blood samples²⁰ and tested for 16 *CFTR* mutations — $\Delta F508$, W1282X, R117H, 621+1(G→T), R334W, R347P, A455E, $\Delta I507$, 1717-1(G→A), G542X, S549N, G551D, R553X, R560T, N1303K, and 3849+10Kb(C→T) — using reverse dot blot strips (Roche Molecular Systems, Alameda, Calif.). DNA was also tested for the G85E mutation.²¹ The length of the sequence of thymidines in intron 8 of the *CFTR* gene was determined with three allele-specific polymerase chain reactions per sample. The common forward primer was 5'TAATGGATCATGGGCCATGT3'. The reverse primers were 5'CCCCAAA-TCCCTGTAAAAAC3', 5'CCCCAAATCCCTGTAAAAAAAAC3' for the 5T, 7T, and 9T alleles, respectively. For each reaction, the dystrophin gene (exon 16) was used as an internal amplification control.²² A sequence of five thymidines in the polyT region is associated with low levels of normal *CFTR* messenger RNA (mRNA).

Tests of the Transepithelial Nasal Potential Difference

The transepithelial nasal potential difference was assessed to evaluate *CFTR*-mediated chloride transport in vivo.²³ Briefly, bioelectric responses were measured during perfusion with solutions containing amiloride, gluconate plus amiloride, and isoproterenol, gluconate, and amiloride to determine the combined effect of removing chloride and activating intracellular cyclic AMP.

Statistical Analysis

Differences between groups were compared with the Wilcoxon rank-sum test.²⁴ All P values are two-tailed. Differences between the expected and observed frequencies of mutations or genotypes were evaluated with a binomial distribution for 54 alleles or 27 patients. This analysis depends on the reliability of data on the prevalence of *CFTR* alleles. The expected frequency of various abnormal alleles was estimated as follows. The frequency of cystic fibrosis among U.S. whites is 1 in 3003.⁶ Among the 2200 white persons who have been genotyped at our medical center, consisting of patients with cystic fibrosis and carriers, the 17 mutations that we tested for in the current study accounted for 75 percent of all alleles causing cystic fibrosis, and half of all patients with cystic fibrosis were homozygous for $\Delta F508$.²⁵ The frequency of the 5T allele is 5 percent among whites tested at our medical center²² and elsewhere.¹⁰⁻¹² Thus, the expected frequency of genotypes consisting of one of the tested mutations on one chromosome and the 5T allele on the other chromosome is 1 in 731 ($2 \times 3003^{-0.5} \times 0.75 \times 0.05$). Finally, patients who were homozygous for $\Delta F508$ would have been excluded from this study because this genotype almost invariably causes clinically significant lung disease. Thus, the expected frequency of genotypes affecting both alleles (carriers of a *CFTR* mutation on one chromosome and the 5T allele on the other [1/731] plus subjects who are homozygous and compound heterozygotes for the tested mutations [$0.75^2/3003$] minus those who are homozygous for $\Delta F508$ [$0.5/3003$]) is 1 in 720 ($1/731 + 0.75^2/3003 - 1/6006$).

RESULTS

Table 1 summarizes the *CFTR* genotypes for the 27 patients with idiopathic pancreatitis. Three different mutations were detected: $\Delta F508$ in five patients, R117H in two, and N1303K in one, for a total of eight. Thus, *CFTR* mutations were found at 11 times

TABLE 1. CHARACTERISTICS OF 27 PATIENTS WITH IDIOPATHIC PANCREATITIS.*

PATIENT No.	SEX	CFTR GENOTYPE	POLYT GENOTYPE	AGE AT DIAGNOSIS	RESULTS OF PANCREATOGRAPHY†
1	M	$\Delta F508/R117H$	9T/7T	45	Moderately abnormal
2	F	$\Delta F508/WT$	9T/5T	32	Moderately abnormal
3	F	$\Delta F508/WT$	9T/5T	48	Moderately abnormal
4	F	$\Delta F508/WT$	9T/7T	40	Moderately abnormal
5	F	$\Delta F508/WT$	9T/7T	15	Mildly abnormal
6	F	R117H/WT	7T/7T	32	Moderately abnormal
7	M	N1303K/WT	7T/9T	43	Moderately abnormal
8	M	WT/WT	5T/7T	33	Moderately abnormal
9	F	WT/WT	5T/7T	29	Normal
10	F	WT/WT	5T/7T	12	Moderately abnormal
11	F	WT/WT	7T/7T	16	Severely abnormal
12	M	WT/WT	7T/7T	22	Mildly abnormal
13	M	WT/WT	7T/7T	31	Normal
14	F	WT/WT	7T/7T	43	Mildly abnormal
15	F	WT/WT	7T/7T	12	Severely abnormal
16	F	WT/WT	7T/7T	54	Moderately abnormal
17	F	WT/WT	7T/7T	47	Moderately abnormal
18	F	WT/WT	7T/7T	65	Mildly abnormal
19	F	WT/WT	7T/7T	12	Not done‡
20	F	WT/WT	7T/7T	59	Moderately abnormal
21	F	WT/WT	7T/7T	42	Mildly abnormal
22	F	WT/WT	7T/7T	33	Severely abnormal
23	F	WT/WT	7T/7T	32	Not done
24	F	WT/WT	7T/7T	54	Mildly abnormal
25	F	WT/WT	7T/7T	54	Severely abnormal
26	F	WT/WT	7T/9T	47	Normal
27	F	WT/WT	7T/9T	21	Severely abnormal

*Boldface type identifies alleles known to cause cystic fibrosis or congenital absence of the vas deferens. WT denotes wild type.

†Pancreatograms were assessed for the severity of chronic pancreatitis according to published criteria.¹⁹ Pancreatograms showing fewer than three abnormal side branches were scored as normal; studies showing at least three abnormal side branches with a normal main pancreatic duct were scored as mildly abnormal; studies showing an abnormal main duct were scored as moderately abnormal; and studies showing additional abnormalities (obstruction, a large cavity, filling defects, or severe dilatation or irregularity) were scored as severely abnormal.

‡In this patient, endoscopic ultrasonography showed a diffusely calcified pancreas, findings consistent with the presence of chronic pancreatitis.

the expected frequency (95 percent confidence interval, 5 to 23; $P < 0.001$) (Table 2).

Patients were also tested for the 5T allele (Fig. 1), and it was present in 5 of the 27 patients. This variant reduces the efficiency of exon 9 splicing and thereby reduces the expression of functional *CFTR* in patients who have a mutation that causes cystic fibrosis on one chromosome and the 5T allele on the other chromosome.^{26,27} Patients with these genotypes resemble patients with cystic fibrosis in that they have congenital absence of the vas deferens, but differ because they do not have lung disease. By contrast, when the 7T or 9T allele is present, there are sufficient levels of properly spliced *CFTR* and these alleles do not cause congenital absence of the vas deferens.

TABLE 2. ASSOCIATION OF ABNORMAL *CFTR* GENOTYPES WITH IDIOPATHIC PANCREATITIS.

VARIABLE	OBSERVED FREQUENCY	EXPECTED FREQUENCY*	PREVALENCE RATIO	95% CONFIDENCE INTERVAL
Mutations causing cystic fibrosis†	8 of 54 alleles	1 of 73 alleles	11:1‡	5–23
5T allele	5 of 54 alleles	1 of 20 alleles	1.9:1	0.8–4.5
Genotypes affecting both <i>CFTR</i> alleles§	3 of 27 patients	1 of 720 patients	80:1‡	17–379

*The expected frequencies are based on four assumptions: that the frequency of cystic fibrosis is 1 in 3003, that the 17 mutations tested for in the study accounted for 75 percent of all mutations causing cystic fibrosis, that the frequency of the 5T allele was 5 percent, and that 50 percent of patients with cystic fibrosis are homozygous for the ΔF508 mutation. The first assumption is based on data for U.S. whites,⁶ and the other three assumptions are based on data for whites tested at our medical center.^{22,25}

†Three mutations causing cystic fibrosis were identified: ΔF508 in five patients, R117H in two, and N1303K in one.

‡P<0.001.

§Two patients had a genotype of ΔF508/wild type, 9T/5T, and one patient had a genotype of ΔF508/R117H, 9T/7T.

A total of 10 patients had *CFTR* mutations or the 5T allele or both (Table 1). None had lung disease typical of cystic fibrosis on the basis of a clinical history or a recent chest film. These 10 patients were 33 years of age on average when pancreatitis was diagnosed and were hospitalized for pancreatitis a total of 116 times (approximately 12 times per patient) during a mean observation period of five years after diagnosis. Nine of the 10 had abnormal findings on pancreatography consistent with a diagnosis of chronic pancreatitis.¹⁹ Four of the 10 (Patients 1, 6, 7, and 8 in Table 1) required pancreatic surgery for pain management. The other 17 patients were similar to these 10 with respect to the age at diagnosis and the severity of pancreatitis.

In three patients both *CFTR* alleles were affected, a frequency that was 80 times the expected rate (95 percent confidence interval, 17 to 379; P<0.001) (Table 2). The genotypes of these three patients (ΔF508/wild type, 9T/5T in two and ΔF508/R117H, 9T/7T in one) are the two most common in patients with congenital absence of the vas deferens.^{10-12,27} These genotypes do not typically cause lung disease. In contrast, lung disease is present in patients with a genotype of ΔF508/R117H, 9T/5T.²⁸

The three patients with abnormalities of both *CFTR* alleles were further evaluated to determine whether they had unrecognized cystic fibrosis-related lung disease (Table 3). None had sweat chloride values diagnostic of cystic fibrosis in adults. Their base-line

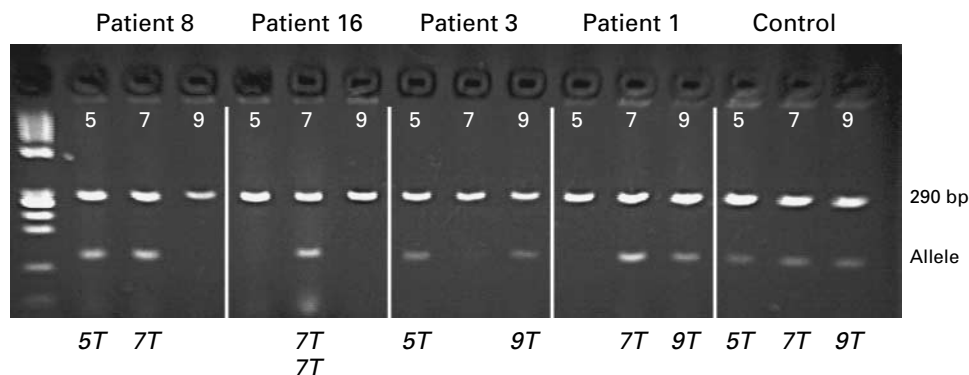


Figure 1. Allele-Specific Polymerase-Chain-Reaction Analysis of the PolyT Sequence of Intron 8 of the *CFTR* Gene. Three reactions were performed for each DNA sample, with specific primers for the 5T, 7T, and 9T alleles (labeled 5, 7, and 9, respectively). Patient 8 had the 5T/7T genotype, Patient 16 had the 7T/7T genotype, Patient 3 had the 5T/9T genotype, Patient 1 had the 7T/9T genotype, and a control sample shows all three alleles. The band at 290 bp in all reactions is an internal amplification control. The first lane shows size markers.

TABLE 3. CHARACTERISTICS OF THREE PATIENTS WITH IDIOPATHIC PANCREATITIS AND TWO ABNORMAL *CFTR* ALLELES.*

PATIENT No.	GENOTYPE	SWEAT CHLORIDE	NASAL POTENTIAL DIFFERENCE	FEV ₁	OTHER FINDINGS
		mmol/liter	mV	% of predicted	
1	$\Delta F508/R117H, 9T/7T$	28, 30	-24	86	Congenital absence of the vas deferens
2	$\Delta F508/WT, 9T/5T$	44, 39	-23	106	Smoker (1 pack/day)
3	$\Delta F508/WT, 9T/5T$	56, 62	-21	58	Smoker (2 packs/day)

*In 20 normal subjects (mean age, 30 years; range, 22 to 41), 13 of whom were male,²³ the sweat chloride concentration was less than 60 mmol per liter and the mean (\pm SD) nasal potential-difference value was -22 ± 7 mV; the respective values in 23 adults with cystic fibrosis were less than 70 mmol per liter and -48 ± 8 mV. FEV₁ denotes forced expiratory volume in one second, and WT wild type.

nasal potential-difference values ranged from -24 to -21 mV, values similar to the mean value of -22 mV in normal adults.²³ Two patients reported no chronic pulmonary symptoms and had normal results on spirometry. In the third patient, the forced expiratory volume in one second was 58 percent of the predicted value. Her sputum contained normal flora, and the abnormal value for forced expiratory volume in one second seemed most consistent with a diagnosis of chronic obstructive pulmonary disease due to cigarette use (2 packs per day). Thus, none of the patients fulfilled traditional diagnostic criteria for the classic cystic fibrosis phenotype.^{5,7,8} However, one of the three patients had congenital absence of the vas deferens, and his sputum contained smooth *Pseudomonas aeruginosa* during an episode of acute bronchitis. (Recovery of either smooth or mucoid strains of *P. aeruginosa* from sputum is unusual during episodes of acute bronchitis in persons who do not have cystic fibrosis; however, the mucoid strain is specifically associated with cystic fibrosis.) Taken together with his genotype, this constellation of findings is suggestive of an atypical cystic fibrosis phenotype.^{5,8}

To assess *CFTR*-mediated ion transport more directly, we measured the nasal potential-difference responses in these three patients. The combined response to the chloride-free maneuver and stimulation by isoproterenol may be a more accurate indicator of *CFTR* function than the sweat test.²³ The changes in values were $+4.5$, $+1.5$, and -5.0 mV in the three patients. These values differed significantly from those measured in 20 normal adults (range, -16 to -44 mV; mean, -31 mV; $P<0.01$) and resembled the values in 23 adults with cystic fibrosis (range, -3 to $+12$ mV; mean, $+5$ mV).²³

DISCUSSION

We found a strong association between chronic pancreatitis and *CFTR* mutations among 27 patients

who were referred for endoscopic evaluation of idiopathic chronic pancreatitis. In these patients, the frequency of a single *CFTR* mutation was 11 times the expected frequency and the frequency of two abnormal alleles was 80 times the expected frequency.

In three patients, both copies of the *CFTR* gene were affected. These patients had the *CFTR* genotypes that are most commonly seen in patients with congenital absence of the vas deferens. In such patients, *CFTR* function is reduced by roughly 90 percent, leading to abnormal nasal chloride transport and congenital absence of the vas deferens but not pancreatic insufficiency, lung disease, or sweat-duct abnormalities.^{7,11} Data on our three patients with idiopathic pancreatitis and these *CFTR* genotypes suggest that their phenotype is similar to that of patients with congenital absence of the vas deferens and these genotypes. Specifically, nasal potential-difference responses of each patient showed defective *CFTR*-mediated ion transport even though none had sweat chloride values diagnostic of cystic fibrosis or evidence of lung disease. Taken together, these findings suggest that in this group of patients, abnormal *CFTR* genotypes cause pancreatitis as one component of an inherited syndrome affecting multiple epithelial tissues and that such patients should be examined for congenital absence of the vas deferens⁹⁻¹² and sinusitis,^{22,29} which are not typical of pancreatitis.

Seven patients had an abnormality in only one copy of the *CFTR* gene. This finding requires cautious interpretation because DNA samples were tested for only 17 of the more than 500 *CFTR* mutations associated with cystic fibrosis.³⁰ Therefore, it is possible that more comprehensive DNA testing (e.g., with single-strand conformation polymorphism analysis) might detect additional mutations. If so, the true magnitude of the association between *CFTR* mutations and pancreatitis was underestimated by our

data. Moreover, it is possible that some of the seven patients may actually be compound heterozygotes with a second mutation that was not detected. Thus, on the basis of our data, we could not determine whether having one copy of an abnormal *CFTR* allele (as is found in carriers of cystic fibrosis) is sufficient to predispose persons to pancreatitis.

Our study calls attention to the relation between *CFTR* genotypes and pancreatitis. Pancreatitis occurs in 1 to 2 percent of patients with cystic fibrosis, with progression to chronic pancreatitis in a minority of these patients.^{5,18} The absence of clinical pancreatitis in most patients with cystic fibrosis is in agreement with the histopathological findings in such patients: there is extensive fibrosis without prominent inflammation.¹⁶ When pancreatitis does develop in patients with cystic fibrosis, lung disease is usually evident. Nonetheless, there have been occasional reports of pancreatitis in patients with cystic fibrosis before the onset of lung disease.^{18,31-34} Pancreatitis occurred before the age of 23 years in each instance, suggesting that pancreatitis may herald the diagnosis of cystic fibrosis in young adults.⁸ Our findings suggest that unexplained pancreatitis in older adults may also be an indicator of mutations in the *CFTR* gene.

Emerging data indicate that cystic fibrosis affects different organs to a varying but predictable extent, depending on the *CFTR* genotype.^{7,8} Genotypes that result in a reduction of functional *CFTR* to 1 percent of normal values cause classic cystic fibrosis consisting of pancreatic insufficiency, lung disease, abnormal sweat chloride concentrations, and congenital absence of the vas deferens. Genotypes that result in a reduction of functional *CFTR* to 5 percent of normal values cause cystic fibrosis without pancreatic insufficiency, consisting of lung disease, abnormal sweat chloride concentrations, and congenital absence of the vas deferens. Genotypes that result in a reduction of functional *CFTR* to 10 percent of normal values cause congenital absence of the vas deferens alone. Thus, one can infer that each manifestation of cystic fibrosis is associated with a reduction of *CFTR* levels below a tissue-specific threshold value (10 percent of normal levels in the case of vas deferens abnormalities, 5 percent in the case of lung and sweat-gland abnormalities, and 1 percent in the case of abnormalities of the exocrine pancreas).

Our data expand on these findings in two respects. First, they suggest that the pancreas and the vas deferens are both relatively susceptible to injury resulting from reduced *CFTR* levels. Thus, pancreatitis and congenital absence of the vas deferens are clinical manifestations of genotypes that cause relatively mild impairment of *CFTR* function. Second, they indicate that the pancreas differs from the lung, sweat gland, and vas deferens in that it is affected in qualitatively different ways depending on the degree to which *CFTR* function is impaired. Severe impair-

ment causes pancreatic insufficiency, and less severe impairment causes pancreatitis. Available evidence suggests that the main role of *CFTR* in the normal human pancreas is to promote the dilution and alkalization of pancreatic juice.¹⁷ Thus, depending on the extent to which *CFTR* function is reduced, either of these two distinct patterns of pancreatic dysfunction can develop.

Our study has implications for the pathogenesis and classification of pancreatitis. It raises the remote possibility that *CFTR* mutations may increase the risk of pancreatitis after exposure to alcohol or certain drugs, and it identifies a subgroup of patients with idiopathic pancreatitis who have abnormalities of both *CFTR* alleles. Since pancreatitis in these patients apparently results from defective *CFTR* function, genetic testing to identify patients at risk may be useful and may increase our understanding of the clinical course of these patients and their response to therapy.

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