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## MUTATIONS OF THE CYSTIC FIBROSIS GENE IN PATIENTS WITH CHRONIC PANCREATITIS

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### ABSTRACT

**Background** The pancreatic lesions of cystic fibrosis develop in utero and closely resemble those of chronic pancreatitis. Therefore, we hypothesized that mutations of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene may be more common than expected among patients with chronic pancreatitis.

**Methods** We studied 134 consecutive patients with chronic pancreatitis (alcohol-related disease in 71, hyperparathyroidism in 2, hypertriglyceridemia in 1, and idiopathic disease in 60). We examined DNA for 22 mutations of the *CFTR* gene that together account for 95 percent of all mutations in patients with cystic fibrosis in the northwest of England. We also determined the length of the noncoding sequence of thymidines in intron 8, since the shorter the sequence, the lower the proportion of normal *CFTR* messenger RNA.

**Results** The 94 male and 40 female patients ranged in age from 16 to 86 years. None had a mutation on both copies of the *CFTR* gene. Eighteen patients (13.4 percent), including 12 without alcoholism, had a *CFTR* mutation on one chromosome, as compared with a frequency of 5.3 percent among 600 local unrelated partners of persons with a family history of cystic fibrosis ( $P < 0.001$ ). A total of 10.4 percent of the patients had the 5T allele in intron 8 (14 of 134), which is twice the expected frequency ( $P = 0.008$ ). Four patients were heterozygous for both a *CFTR* mutation and the 5T allele. Patients with a *CFTR* mutation were younger than those with no mutations ( $P = 0.03$ ). None had the combination of sinopulmonary disease, high sweat electrolyte concentrations, and low nasal potential-difference values that are diagnostic of cystic fibrosis.

**Conclusions** Mutations of the *CFTR* gene and the 5T genotype are associated with chronic pancreatitis. (N Engl J Med 1998;339:645-52.)

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IN 1969, Paris et al.<sup>1</sup> described two siblings who had the autosomal recessive disease cystic fibrosis and whose father and paternal uncle and grandfather had chronic calcific pancreatitis. Not only did this report hint at a shared molecular basis for pancreatic damage in these two conditions,<sup>2</sup> but it also, since the children's mother was apparently unaffected, underlined the importance of nature-nurture interactions in the pathogenesis of the sporadic form of chronic pancreatitis.<sup>3</sup>

The presentation of chronic pancreatitis typically resembles that of acute pancreatitis; subsequent attacks can be anticipated until all secretory parenchyma is destroyed. Alcoholism is a major etiologic factor, exposure to cigarette smoke and occupational exposure to volatile hydrocarbons independently increase the risk,<sup>4,5</sup> duct-obstructing lesions initiate a few cases, and there may also be an underlying metabolic or autoimmune disorder. The cause of the rare hereditary form has been identified as a mutation in the cationic trypsinogen gene at locus 7q35.<sup>6</sup> The disease is idiopathic in up to 40 percent of affected patients in developed countries.

The exocrine pancreas is invariably affected in cystic fibrosis.<sup>7</sup> The lesion has been described as "basically a diffuse form of chronic pancreatitis."<sup>8</sup> The damage begins in utero<sup>7</sup> and can be identified in neonates on the basis of elevated blood concentrations of pancreatic enzymes, classically trypsinogen. Pancreatic biopsy in the first year of life reveals interstitial inflammation,<sup>9</sup> but this is not found on postmortem examination after the failure of pancre-

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atic exocrine function. The progression of the disease is usually rapid and painless; however, a few patients may have an attack of pancreatitis or pancreatic calculi.

In 1989, the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene was identified at locus 7q31.<sup>10</sup> This discovery led to the suggestion that insufficiency of the *CFTR* protein may underlie the overlapping clinicopathological facets of chronic pancreatitis and cystic fibrosis.<sup>2</sup> As a first step toward testing this hypothesis, we examined the frequency of *CFTR* mutations in a cohort of patients with chronic pancreatitis.

## METHODS

### Study Design

In a study of 600 unrelated partners of persons with a family history of cystic fibrosis in the northwest of England, the rate of carriage of *CFTR* mutations was 5.3 percent (95 percent confidence interval, 3.5 to 7.1 percent).<sup>11</sup> Therefore, we calculated that a minimum of 106 patients with chronic pancreatitis would have to be examined for the study to be able to detect, at a power of 90 percent, a doubling of this frequency. Study of the attendance register at the weekly pancreatobiliary clinic indicated that this target could be met within six months. We decided at the outset that patients who were found to have a *CFTR* mutation would undergo supplementary tests for atypical cystic fibrosis<sup>12</sup> and that their first-degree relatives would be offered the opportunity to undergo screening for *CFTR* mutations.<sup>11</sup>

### Patients

The study was approved by the hospital's ethics committee. Between January 1993 and June 1993, consecutive white patients with chronic pancreatitis were enrolled after they gave informed consent. The diagnosis of chronic pancreatitis was based on standard criteria: abnormalities on histologic analysis of biopsy specimens, visible calculi on x-ray films, unequivocally abnormal findings on endoscopic pancreatography,<sup>13</sup> or impaired exocrine secretory capacity, determined by the secretin-pancreozymin test (bicarbonate or enzyme output more than 2 SD below the mean in normal subjects)<sup>13</sup> or the *p*-aminobenzoic acid excretion index (results more than 3 SD below the mean in normal subjects in our version of this tubeless test, which uses bentrimide).<sup>14,15</sup> Patients with a periampullary lesion that obstructed duct drainage were excluded.

For the purposes of the study, alcoholism was defined as the daily intake of at least 80 g of ethanol by men and at least 60 g of ethanol by women for two years before the first symptom of pancreatitis, and cigarette smokers were defined as those who smoked 10 or more cigarettes per day.<sup>5</sup> Those who drank lower amounts of alcohol were classified as nonalcoholics, and those who smoked zero to nine cigarettes per day were classified as non-smokers. Job histories were also available, but we did not analyze these data because of the difficulty of quantifying occupational exposure to hydrocarbon.<sup>6</sup> All patients were taking antioxidant supplements to control pain, and most had been taking them for about five years. The rationale for antioxidant therapy has been discussed previously.<sup>3,16-18</sup> A frequent starting regimen consisted of six tablets containing organic selenium, beta carotene, and vitamins C and E (Wassen, Leatherhead, United Kingdom), and eight tablets of methionine (Evans Medical, Horsham, United Kingdom) per day in divided doses, for total daily supplements of 600  $\mu$ g of organic selenium, 9000 IU of beta carotene, 0.54 g of vitamin C, 270 IU of vitamin E, and 2 g of methionine. Doses were adjusted after periodic measurement of blood vitamin C, selenium, and glutathione.

### DNA Studies

We extracted DNA from buccal cells obtained by having the patients rinse their mouths with 10 ml of 4 percent sucrose.<sup>19</sup> The *CFTR* locus was examined for the 22 mutations that together account for 95 percent of all such mutations in patients with cystic fibrosis in the northwest of England.<sup>20</sup> The amplification-refractory mutation system Elucigene CF(4)m kit (Zeneca Diagnostics, Macclesfield, United Kingdom) was used to detect the four most common mutations:  $\Delta$ F508, G551D, G542X, and 621+1(G $\rightarrow$ T)<sup>21</sup>; the polymerase chain reaction, restriction-enzyme analysis, and allele-specific oligonucleotide hybridization facilitated the detection of R560T, R117H, 1898+1(G $\rightarrow$ A), R553X, S549N, 1717-1(G $\rightarrow$ A), N1303K, W1282X, E60X, 1154insTC, R347P, 3659delC, Q493X, V520E, R334W,  $\Delta$ 1507, 3849+10Kb(C $\rightarrow$ T), and 1078delT. Low levels of *CFTR* protein may result from a reduction in normal messenger RNA (mRNA), such as is associated with a mutation in the noncoding sequence of thymidines in intron 8.<sup>22,23</sup> This sequence may contain five, seven, or nine thymidines (the 5T, 7T, and 9T alleles, respectively); the shorter the sequence, the lower the proportion of normal *CFTR* mRNA. The length of the intron 8 polyT region was determined by oligonucleotide hybridization<sup>22</sup> or direct sequencing.

### Assessment for Atypical Cystic Fibrosis

Pulmonary spirometry and sinopulmonary radiography were performed whenever possible. Studies of the transepithelial nasal potential difference<sup>24</sup> were undertaken in a subgroup of patients with a *CFTR* mutation and in comparable groups of patients with chronic pancreatitis and normal subjects with no *CFTR* mutations. The test involves the perfusion of a nasal mucosal electrode with standard buffer, then with 100  $\mu$ M amiloride in standard buffer to block the epithelial sodium channel, then with 100  $\mu$ M amiloride in low-chloride buffer to stimulate chloride movement, and finally with 100  $\mu$ M amiloride and 10  $\mu$ M isoproterenol in low-chloride buffer to increase intracellular cyclic AMP. Pilocarpine iontophoresis<sup>25</sup> was used to obtain sweat samples from the same subgroups; a minimum of 100 mg of sweat was analyzed to determine sodium and chloride concentrations. Several of the younger patients had already undergone sweat testing at their initial presentation.

### Statistical Analysis

A chi-square statistic with Yates' correction, a binomial distribution, the Mann-Whitney U test, and Fisher's exact test were used as appropriate. All P values were two-tailed. A P value of less than 0.05 was considered to indicate statistical significance.<sup>26</sup>

## RESULTS

### Characteristics of the Patients

The cohort of 134 patients included 71 with alcohol-related pancreatitis, 3 with a metabolic problem (2 with hyperparathyroidism and 1 with severe hypertriglyceridemia), and 60 with idiopathic pancreatitis. There were 94 male and 40 female patients. The age at onset of symptoms varied widely, from 5 to 81 years, as did age at the time of the study (16 to 86 years). Ninety-nine patients smoked 10 or more cigarettes a day. Large-duct disease was identified on the basis of an abnormal pancreatogram or radiographic evidence of calculi in 80 percent of the patients, and small-duct disease was identified on the basis of histologic findings or impaired exocrine function in the others. An attack of pancreatitis was the usual presenting symptom, and increasing pain was the usual reason for referral.

## DNA Studies

No patient had a mutation on both copies of the *CFTR* gene. Eighteen patients (13.4 percent; 95 percent confidence interval, 8.2 to 20.4 percent) had a *CFTR* mutation on one chromosome, as compared with 32 of the 600 unrelated partners of persons with a family history of cystic fibrosis (5.3 percent; 95 percent confidence interval, 3.5 to 7.1 percent;  $P < 0.001$ ). The most common mutation was  $\Delta F508$  (Table 1), as is the case among patients with cystic fibrosis in the northwest of England.<sup>20</sup> The group of 18 patients with a *CFTR* mutation were younger at presentation than the other 116 patients ( $P = 0.03$ ) and included 12 nonalcoholics. There was a higher frequency of *CFTR* mutations among patients who were classified as nonsmokers than among those classified as smokers (28.6 percent vs. 8.1 percent,  $P = 0.007$ ), but there was no significant difference in the frequency of mutations between alcoholics and nonalcoholics (8.5 percent vs. 19.0 percent,  $P = 0.12$ ).

Analysis of the polyT sequence identified the 5T allele in 14 of 134 patients, or 10.4 percent (95 per-

cent confidence interval, 5.8 to 16.9 percent); the frequency is 5.0 percent in the general population<sup>22</sup> ( $P = 0.008$ ). It was present in 4 of the 18 patients with a *CFTR* mutation and in 10 (all males) of the other 116 patients (22.2 percent vs. 8.6 percent,  $P = 0.10$ ). The clinicopathological features of the patients with chronic pancreatitis classified according to whether they had a 5T allele or a *CFTR* mutation alone or in combination are summarized in Table 2. On the basis of the work of Chillón et al.,<sup>27</sup> it is likely that the *CFTR* mutation and the 5T allele were on opposite chromosomes.

## Assessment for Atypical Cystic Fibrosis

None of the 18 patients with a *CFTR* mutation alone or in combination with a 5T allele met the diagnostic criteria for cystic fibrosis when all the evidence was considered.<sup>12</sup> A review of family histories revealed cystic fibrosis in close relatives of two unrelated patients. Subsequent screening identified the disease in the outwardly healthy infant son of another patient.

There were no sinopulmonary symptoms or signs

TABLE 1. CHARACTERISTICS OF 18 PATIENTS WITH CHRONIC PANCREATITIS AND A MUTANT *CFTR* ALLELE.\*

| PATIENT NO.† | SEX | MUTANT ALLELE | POLYT GENOTYPE | AGE AT ONSET OF PANCREATITIS | AGE AT STUDY ENTRY | EXOCRINE STATUS AND CALCULI‡ | ALCOHOLISM | ≥10 CIGARETTES/DAY | SWEAT TESTING |          | BASE-LINE NASAL POTENTIAL DIFFERENCE |
|--------------|-----|---------------|----------------|------------------------------|--------------------|------------------------------|------------|--------------------|---------------|----------|--------------------------------------|
|              |     |               |                |                              |                    |                              |            |                    | SODIUM        | CHLORIDE |                                      |
|              |     |               |                | yr                           |                    |                              | mmol/liter |                    | mV            |          |                                      |
| 1            | M   | $\Delta F508$ | 9T/7T          | 8                            | 27                 | PS0                          | No         | No                 | 43.5          | 32.0     | 12.5                                 |
| 2            | F   | $\Delta F508$ | 9T/5T          | 15                           | 34                 | PS1                          | No         | No                 | 55.0          | 47.5     | ND                                   |
| 3            | M   | R117H         | 7T/7T          | 18                           | 21                 | PS0                          | No         | Yes                | 44.0          | 33.0     | -9.7                                 |
| 4            | M   | $\Delta F508$ | 9T/7T          | 18                           | 26                 | PI3                          | No         | No                 | ND            | ND       | ND                                   |
| 5            | M   | $\Delta F508$ | 9T/7T          | 18                           | 30                 | PI3                          | No         | Yes                | ND            | ND       | ND                                   |
| 6            | F   | Q493X         | 7T/5T          | 19                           | 21                 | PS3                          | No         | Yes                | 51.5          | 41.0     | ND                                   |
| 7            | F   | $\Delta F508$ | 9T/7T          | 20                           | 31                 | PS3                          | No         | No                 | 35.0          | 23.0     | -10.8                                |
| 8            | M   | 621+1(G→T)    | 9T/7T          | 21                           | 37                 | PS3                          | Yes        | Yes                | 72.0          | 48.5     | 5.0                                  |
| 9            | M   | R560T         | 7T/7T          | 21                           | 39                 | PI0                          | Yes        | Yes                | 103.0         | 76.0     | -4.4                                 |
| 10           | M   | $\Delta F508$ | 9T/5T          | 22                           | 36                 | PI3                          | Yes        | No                 | 53.0          | 34.0     | -17.6                                |
| 11           | M   | $\Delta F508$ | 9T/7T          | 31                           | 45                 | PS3                          | No         | Yes                | 55.0          | 34.0     | -11.5                                |
| 12           | M   | R117H         | 7T/7T          | 35                           | 38                 | PI2                          | Yes        | No                 | ND            | ND       | ND                                   |
| 13           | F   | $\Delta F508$ | 9T/7T          | 36                           | 39                 | PS3                          | No         | Yes                | 60.0          | 39.0     | -10.2                                |
| 14           | F   | R553X         | 7T/5T          | 37                           | 56                 | PI3                          | No         | Yes                | ND            | ND       | ND                                   |
| 15           | F   | $\Delta F508$ | 9T/7T          | 45                           | 47                 | PI3                          | Yes        | Yes                | 104.0         | 80.0     | -8.3                                 |
| 16           | M   | $\Delta F508$ | 9T/7T          | 49                           | 52                 | PS1                          | Yes        | Yes                | ND            | ND       | ND                                   |
| 17           | F   | $\Delta F508$ | 9T/7T          | 64                           | 76                 | PI3                          | No         | No                 | 69.0          | 50.0     | -10.3                                |
| 18           | F   | $\Delta F508$ | 9T/9T          | 75                           | 79                 | PS3                          | No         | No                 | 34.5          | 19.0     | -14.7                                |

\*Large-duct disease was identified in all but Patient 3, who had small-duct disease with nearly normal findings on pancreatography, a value of 0.62 on the *p*-aminobenzoic acid excretion index (mean  $-3$  SD, 0.75), and confirmatory histologic findings. ND denotes not done.

†Patient 1 had azoospermia. The baby son of Patient 7 was identified as having cystic fibrosis on subsequent screening. The nephew of Patient 8 and the niece of Patient 18 had cystic fibrosis.

‡PS denotes pancreatic sufficiency, defined as a value above 0.3 on the *p*-aminobenzoic acid excretion index, and PI pancreatic insufficiency, defined as a value of 0.3 or less on the *p*-aminobenzoic acid excretion index. A score of zero indicates no pancreatic calculi, a score of one an isolated focus, a score of two sparse calculi detected only by computed tomography, and a score of three dense and widespread calculi evident on abdominal x-ray films.

**TABLE 2.** CHARACTERISTICS OF PATIENTS WITH CHRONIC PANCREATITIS ACCORDING TO WHETHER THEY HAD A *CFTR* MUTATION OR A *5T* ALLELE ALONE OR IN COMBINATION.

| CHARACTERISTIC                          | NORMAL<br><i>CFTR</i> GENES<br>(N=106) | <i>5T</i> ALLELE<br>ALONE (N=10) | <i>CFTR</i> MUTATION<br>WITH OR WITHOUT<br>A <i>5T</i> ALLELE (N=18) | <i>CFTR</i> MUTATION<br>ALONE (N=14) | <i>CFTR</i> MUTATION<br>AND A <i>5T</i> ALLELE<br>(N=4) |
|---|--|----------------------------------|--|--------------------------------------|---|
| Sex — M/F                               | 74/32                                  | 10/0                             | 10/8   | 9/5                                  | 1/3   |
| Age at presentation — yr                |  |                                  |  |                                      |   |
| Mean                                    | 34                                     | 26                               | 21   | 26                                   | 20  |
| Range                                   | 5–81                                   | 10–51                            | 8–75   | 8–75                                 | 15–37   |
| Age at study — yr                       |  |                                  |  |                                      |   |
| Mean                                    | 48                                     | 41                               | 37   | 38                                   | 35  |
| Range                                   | 19–86                                  | 16–60                            | 21–79  | 21–79                                | 21–59   |
| Score for pancreatic calculi — no. (%)* |  |                                  |  |                                      |   |
| 0                                       | 23 (22)                                | 4 (40)                           | 3 (17)   | 3 (21)                               | 0   |
| 1                                       | 13 (12)                                | 1 (10)                           | 3 (17)   | 2 (14)                               | 1 (25)  |
| 2                                       | 17 (16)                                | 1 (10)                           | 0  | 0                                    | 0   |
| 3                                       | 53 (50)                                | 4 (40)                           | 12 (67)  | 9 (64)                               | 3 (75)  |
| Exocrine status — no. (%)†              |  |                                  |  |                                      |   |
| Pancreatic sufficiency                  | 62 (58)                                | 9 (90)                           | 10 (56)  | 8 (57)                               | 2 (50)  |
| Pancreatic insufficiency                | 44 (42)                                | 1 (10)                           | 8 (44)   | 6 (43)                               | 2 (50)  |
| Endocrine status — no. (%)              |  |                                  |  |                                      |   |
| Normal                                  | 64 (60)                                | 9 (90)                           | 10 (56)  | 8 (57)                               | 2 (50)  |
| Impaired glucose tolerance              | 14 (13)                                | 0                                | 1 (6)  | 0                                    | 1 (25)  |
| Drug-controlled diabetes                | 7 (7)                                  | 0                                | 3 (17)   | 2 (14)                               | 1 (25)  |
| Insulin-dependent diabetes              | 21 (20)                                | 1 (10)                           | 4 (22)   | 4 (29)                               | 0   |
| Etiologic factors — no. (%)‡            |  |                                  |  |                                      |   |
| Alcohol                                 | 60 (57)                                | 5 (50)                           | 6 (33)   | 5 (36)                               | 1 (25)  |
| Cigarettes                              | 83 (78)                                | 6 (60)                           | 10 (56)  | 8 (57)                               | 2 (50)  |

\*A score of zero indicates no pancreatic calculi, a score of one an isolated focus, a score of two sparse calculi detected only by computed tomography, and a score of three dense and widespread calculi evident on abdominal x-ray films.

†Pancreatic sufficiency was defined as a value above 0.3 on the *p*-aminobenzoic acid excretion index, and pancreatic insufficiency was defined as a value of 0.3 or less on the *p*-aminobenzoic acid excretion index.

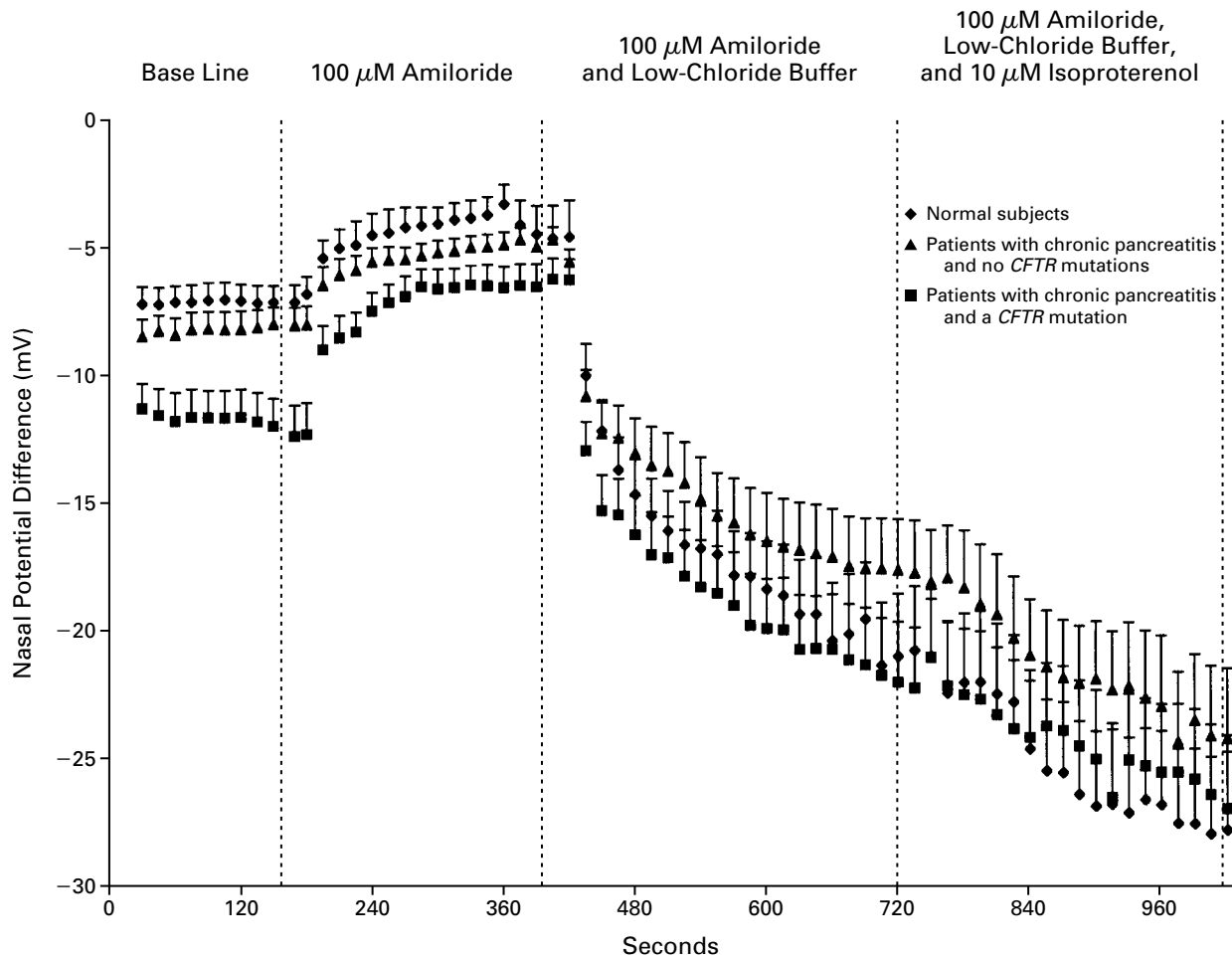
‡There were no significant differences in the proportions of alcoholics or cigarette smokers between the various subgroups. Thirty-three percent of patients with a *CFTR* mutation and 56 percent of those without a *CFTR* mutation were alcoholics ( $P=0.08$  by two-tailed Fisher's exact test); 55 percent of patients with a *CFTR* mutation and 77 percent of those without such a mutation smoked  $\geq 10$  cigarettes per day ( $P=0.10$ ).

or radiologic abnormalities in 133 patients. The one exception was a 76-year-old woman (Patient 17 in Table 1) who was a former smoker with the  $\Delta F508/-(9T/7T)$  genotype and mild bronchiectasis but without colonization by pseudomonas strains. Spirometry was possible in 109 patients, including all 18 with a *CFTR* mutation. Evidence of obstruction (ratio of forced expiratory volume in one second to forced vital capacity,  $<70$  percent) was found in 4 of the 18 patients with a *CFTR* mutation (22.2 percent) and in 23 of 91 patients with no *CFTR* mutations (25.3 percent). The former group included three patients who smoked at least 10 cigarettes daily (Patients 14, 15, and 16 in Table 1) and a non-smoker with a history of hyperparathyroidism (Patient 17 in Table 1).

Nasal potential-difference tests (Fig. 1) were interpreted with reference to published studies,<sup>24</sup> after we confirmed that the pattern was abnormal in patients with classic cystic fibrosis by testing two such patients (data not shown). As compared with the mean ( $\pm$ SE) value in 12 normal subjects with no *CFTR* mutations ( $-7.2 \pm 0.7$ ), the base-line value was significantly lower in the subgroup of patients with a

*CFTR* mutation ( $-10.5 \pm 1.2$ ,  $P=0.02$ ) but not in the subgroup with no *CFTR* mutations ( $-8.1 \pm 0.5$ ). However, no patient had a value that was diagnostic of cystic fibrosis (approximately  $-50$  mV)<sup>12</sup> and the results in our two patients with cystic fibrosis were close to this value ( $-48$  and  $-56$  mV). Moreover, the patterns and gradients of responses to the various perfusates were similar in the three subgroups. Only one of the four patients who were heterozygous for both a *CFTR* mutation and the *5T* allele agreed to be tested (Patient 10 in Table 1). His baseline value was the lowest recorded ( $-17.6$  mV), and it changed to  $-11.8$  mV after exposure to amiloride — representing a change of 32.9 percent, as compared with approximately 70 percent in patients with cystic fibrosis<sup>12</sup> — and changed to  $-20.7$  mV with a low-chloride perfusate and to  $-22.5$  mV after exposure to isoproterenol.

Sweat tests showed a stepwise increase in electrolyte concentrations, with the normal subjects having the lowest concentrations and the patients with a *CFTR* mutation the highest concentrations (Fig. 2). There was no significant change in electrolyte concentrations in five patients who were examined twice,



**Figure 1.** Mean (+SE) Values for Nasal Potential-Difference Measurements in 12 Normal Subjects, 12 Patients with Chronic Pancreatitis and No *CFTR* Mutations, and 11 Patients with Chronic Pancreatitis and a *CFTR* Mutation.

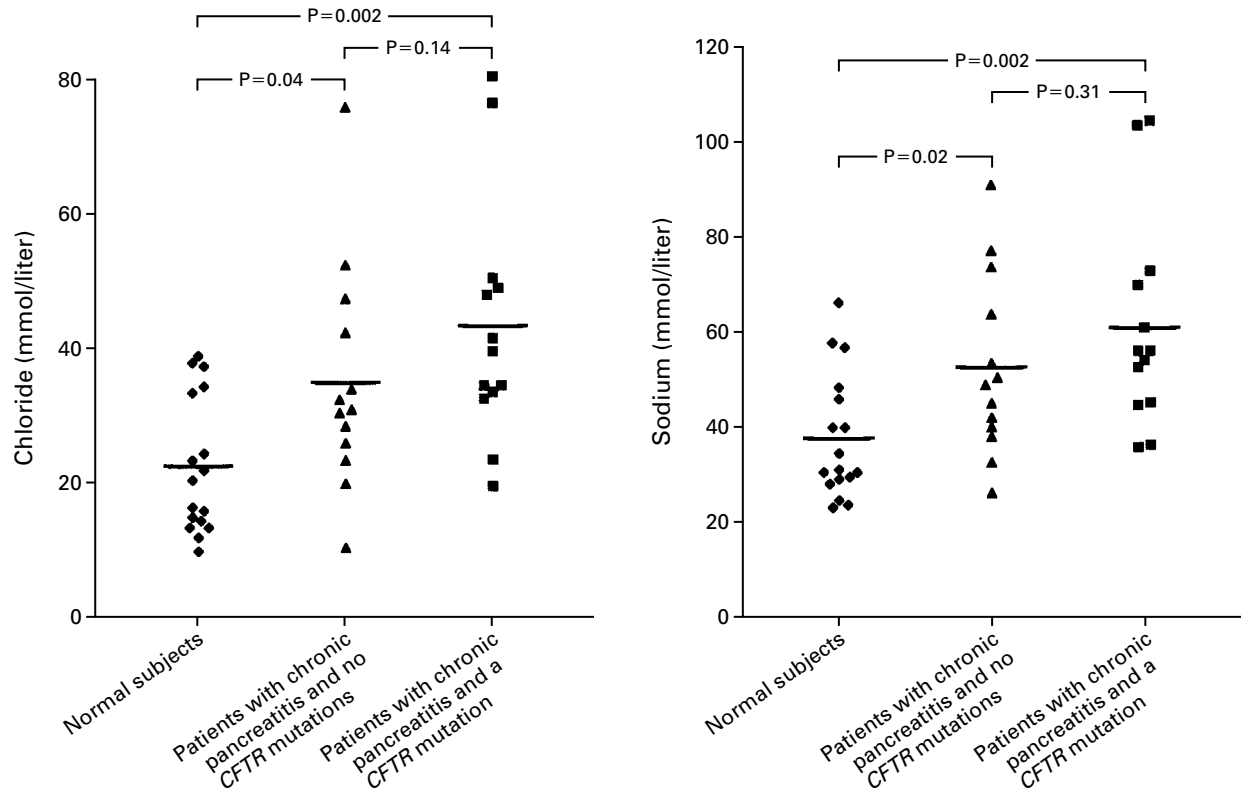
The normal subjects were 8 men and 4 women with a median age of 37 years (range, 20 to 64), the 12 patients with chronic pancreatitis and no *CFTR* mutations were 8 men and 4 women with a median age of 47 (range, 23 to 68), and the 11 patients with chronic pancreatitis and a *CFTR* mutation were 6 men and 5 women with a median age of 39 (range, 21 to 79). The points represent readings taken at 15-second intervals with the mucosal electrode initially perfused with Krebs' HEPES buffer to obtain a base-line reading, then with 100  $\mu$ M amiloride in standard buffer, amiloride in low-chloride buffer, and amiloride together with 10  $\mu$ M isoproterenol in low-chloride buffer. There is a brief disjunction of data points 30 to 45 seconds after the perfusion of each solution because this interval equates to the dead space of the equipment and the response time of the epithelium. As compared with the value in the normal subjects ( $-7.2 \pm 0.7$ ), the base-line value was significantly lower in the patients with a *CFTR* mutation ( $-10.5 \pm 1.2$ ,  $P=0.02$ ) but not in the patients with no *CFTR* mutations ( $-8.1 \pm 0.5$ ).

at presentation and again for the study, while receiving antioxidant therapy. A sweat chloride concentration of at least 60 mmol per liter, which is suggestive of cystic fibrosis,<sup>12</sup> was found in three patients, one with a normal *CFTR* genotype and two with a *CFTR* mutation but normal results on spirometry and nasal potential-difference tests (Patients 8 and 15 in Table 1). Alcoholism, but not cigarette smoking, as defined in this study, may have contributed to this outcome (mean chloride concentration, 51 mmol per liter in eight patients with alcoholism and 37 mmol per liter in 18 patients without alcoholism, irrespective of the *CFTR* genotype;  $P=0.02$ ).

Male patients with cystic fibrosis frequently have azoospermia,<sup>12</sup> as was found in a patient (Patient 1 in Table 1) who has been described previously in another context.<sup>28</sup> Of the other nine male patients, four are fathers, one had normal results on semen analysis, and four declined to undergo semen analysis.

#### ***CFTR* Genotype and the Pancreatic Phenotype**

Among patients with cystic fibrosis, mutations have been described that result in pancreatic insufficiency (e.g.,  $\Delta F508$ ), necessitating enzyme supplementation, or in disease that does not affect pancreatic function to the same extent (e.g., R117H),<sup>29</sup>



**Figure 2.** Sweat Chloride and Sodium Concentrations in 17 Normal Subjects, 13 Patients with Chronic Pancreatitis and No *CFTR* Mutations, and 13 Patients with Chronic Pancreatitis and a *CFTR* Mutation.

The normal subjects were eight men and nine women with a median age of 36 years (range, 20 to 64), the patients with chronic pancreatitis and no *CFTR* mutations were nine men and four women with a median age of 45 (range, 23 to 68), and the patients with chronic pancreatitis and a *CFTR* mutation were six men and seven women with a median age of 39 (range, 21 to 79). The horizontal lines indicate the means.

but no such clinical differences were discernible in our patients (Table 2). We do not know whether antioxidant supplementation, which controlled pain sufficiently in all but one patient, who underwent distal pancreatectomy after referral because a tumor was thought to be present, altered the natural history of chronic pancreatitis.<sup>3</sup>

### DISCUSSION

Our investigation had three linked objectives: to assess whether, because certain features of pancreatic involvement overlap in chronic pancreatitis and cystic fibrosis,<sup>2</sup> *CFTR* mutations are more common than would be expected among patients with chronic pancreatitis; to determine whether patients with a mutation had an atypical form of cystic fibrosis<sup>12</sup>; and to seek clues to the possible role of *CFTR* mutations in the development of chronic pancreatitis.<sup>3</sup>

In a cohort of 134 patients, we found that the frequency of a *CFTR* mutation was nearly 2.5 times as high as expected and that the frequency of the 5T allele was twice as high as expected. *CFTR* mutations were associated with idiopathic rather than al-

cohol-related disease, as was the case in preliminary reports by others,<sup>30-32</sup> and there was a high rate of mutations among nonsmokers or those who smoked fewer than 10 cigarettes a day. A diagnosis of atypical cystic fibrosis was not justified in these patients,<sup>12</sup> but a few patients had a partial pattern of extrapancreatic involvement compatible with this diagnosis, and a more extensive molecular genetic analysis might have identified other such patients. However, on the basis of our findings, we conclude that chronic pancreatitis should be added to the list of conditions in which a mutant *CFTR* gene has pathogenetic importance. This list includes congenital absence of the vas deferens,<sup>27</sup> nasal polyposis,<sup>33</sup> diffuse bronchiectasis,<sup>34</sup> and bronchopulmonary allergic aspergillosis in adults.<sup>35</sup>

There is a fundamental difference between our findings and those reported in patients with congenital absence of the vas deferens.<sup>27</sup> Among patients with congenital absence of the vas deferens, 19 percent had a *CFTR* mutation in both copies of the gene without a 5T allele on either chromosome (a genotype that is calculated to reduce the percent-

age of normal CFTR mRNA to less than 3 percent of normal), and 34 percent had a 5T allele with a CFTR mutation on the opposite chromosome or were 5T homozygotes (genotypes that would result in a reduction of functional CFTR mRNA to between 8 and 12 percent of normal).<sup>23,27</sup> In contrast, none of our patients with chronic pancreatitis had two CFTR mutations, 11 percent had a CFTR mutation and no 5T allele, and 3 percent had both a CFTR mutation and a 5T allele. These data suggest that CFTR mutations are a sufficient explanation for the problem with the development of the vas deferens in at least 50 percent of affected persons but that the relation between CFTR mutations and the development of chronic pancreatitis is more subtle.

Ductal obstruction is generally regarded as the initiating event in both chronic pancreatitis and cystic fibrosis. However, this theory is undermined by several observations,<sup>2,3,13,36,37</sup> as well as by histologic evidence to the contrary.<sup>7-9,28</sup> We favor an alternative explanation wherein the acinar cell is a direct target<sup>2,3,38</sup> and the damage is amplified when bicarbonate-producing epithelium is affected in a manner that reduces the pH within the intra-acinar space and lumen of ductules.<sup>39</sup> The involvement of the CFTR protein in intracellular-vesicle targeting, movement of macromolecules, and membrane recycling,<sup>37,40</sup> over and above its role in ion transport, is central to this concept.

The sweat studies in our patients were not meant to be definitive but rather to document the findings in a newly characterized subgroup with a CFTR mutation, since increased sweat electrolyte concentrations have been reported in patients with alcohol-related chronic pancreatitis.<sup>41,42</sup> We found that the electrolyte concentrations were independent of CFTR mutations, although concentrations were higher in patients with a mutation (Fig. 2). It is impossible to assess the meaning of the lower base-line values for nasal potential difference in the patients with a CFTR mutation, because several ion channels contribute to the result.<sup>43</sup>

In conclusion, our study identifies mutations of the CFTR gene as a risk factor for chronic pancreatitis. Further studies are needed to explain why chronic pancreatitis does not develop in the majority of persons with a CFTR mutation and to examine the relation between a CFTR mutation and a mutation in the cationic trypsinogen gene.<sup>44</sup>

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## CORRECTION

**Correction: Mutations of the Cystic Fibrosis Gene in Patients with Chronic Pancreatitis**

*To the Editor:* In their article on mutations of the cystic fibrosis gene in patients with chronic pancreatitis, Sharer et al. (Sept. 3 issue)<sup>1</sup> concluded that the frequency of carriers of the 5T allele was significantly higher in these patients than in the general population. However, we believe that this conclusion may be incorrect. The authors did not use for comparison the control group of 600 unrelated partners of persons with a family history of cystic fibrosis in the northwest of England.<sup>2</sup> Rather, they used the series studied by Kiewewetter et al.,<sup>3</sup> which included persons from the United States, Northern Ireland, Italy, and Greece. The control group should represent the population from which the case patients were selected. Moreover, the carrier rate of 10.4 percent for the 5T allele found among 134 patients with chronic pancreatitis was compared with a 5 percent prevalence among 224 normal chromosomes (143 from the parents of patients with cystic fibrosis and 81 from the general population).<sup>3</sup> The comparison of the carrier prevalence rate with the allele prevalence rate is inappropriate because the former is approximately two times as high, since a person has two homologous chromosomes. Thus, their conclusion that the frequency of the 5T allele among patients with chronic pancreatitis is "twice as high as expected" is incorrect. To date, there is no evidence that the 5T allele confers susceptibility to chronic pancreatitis.<sup>4</sup>

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The authors reply:

*To the Editor:* The length of the thymidine-repeat sequence in intron 8 of the cystic fibrosis gene (the polyT sequence) may be five, seven,

or nine bases. The frequency with which these three alleles occur in a white population does not differ among studies,<sup>1,2,3,4</sup> with values of 5, 84, and 11 percent, respectively, being reported in the 224 normal chromosomes analyzed by Kiewewetter et al.<sup>1</sup> In our study of mutations of the cystic fibrosis gene in patients with chronic pancreatitis, we assumed that these figures would apply equally to the population in the northwest of England.

A total of 10.4 percent of the 134 patients with chronic pancreatitis whom we studied carried the 5T allele. We are grateful to Drs. Malats and Real for bringing to our attention that we incorrectly compared this carrier rate with a 5T allele frequency rate in the population of 5 percent. We acknowledge our error. In our conclusions, we should have stated that mutations of the CFTR gene — but not the 5T genotype — are associated with chronic pancreatitis. We agree that, unlike the case with congenital absence of the vas deferens<sup>4</sup> and disseminated bronchiectasis,<sup>2</sup> a relation between susceptibility to chronic pancreatitis and the presence of a 5T allele remains unproved. This point exemplifies the differences among these three cystic fibrosis syndromes<sup>5</sup> and the as yet undefined interactions between genotype and environmental factors in their causation.

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