THE RELATION BETWEEN GENOTYPE AND PHENOTYPE IN CYSTIC FIBROSIS — ANALYSIS OF THE MOST COMMON MUTATION (ΔF_{508})

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Abstract *Background and Methods.* Both the clinical manifestations of cystic fibrosis and the genotypes of patients are heterogeneous, but the associations between the two are not known. We therefore studied blood samples from 293 patients with cystic fibrosis for the presence of the most common disease-causing mutation (ΔF_{508}) on chromosome 7 and compared the results with the clinical manifestations of the disease.

Results. The prevalence of the ΔF_{508} allele in the cohort was 71 percent; 52 percent of the patients were homozygous for the mutation, 40 percent were heterozygous, and 8 percent had other, undefined mutations. The patients who were homozygous for the mutation had received a diagnosis of cystic fibrosis at an earlier age and had a greater frequency of pancreatic insufficiency; pancreatic insufficiency was present in 99 percent of the homozygous patients, but in 72 percent of the heterozygous patients and only 36 percent of the patients with other genotypes. The patients with pancreatic insufficiency in all three genotype groups had similar clinical characteristics, reflected by an early age at diagnosis, similar sweat chloride values at diagnosis, similar severity of pulmonary disease, and similar percentiles for weight. In contrast, the patients in the heterozygous-genotype and other-genotype groups who did not have pancreatic insufficiency were older and had milder disease. They had lower sweat chloride values at diagnosis, normal nutritional status, and better pulmonary function after adjustment for age.

Conclusions. The variable clinical course in patients with cystic fibrosis can be attributed at least in part to specific genotypes at the locus of the cystic fibrosis gene. (N Engl J Med 1990; 323:1517-22.)

CYSTIC fibrosis is an inherited disorder characterized by progressive lung disease, pancreatic insufficiency, impaired growth, elevated sweat electrolyte values, and other, less common clinical findings, including meconium ileus, nasal polyposis, and hepatobiliary disease.[1] The presentation varies at different ages, and the severity of disease and its rate of progression in the involved organs vary considerably. Although most patients have pancreatic insufficiency that necessitates exogenous enzyme-replacement therapy, approximately 15 percent have enough residual exocrine pancreatic function to permit normal digestion without enzyme supplements[2] (although enzyme secretion ranges from normal to 1 percent of the mean normal value[3]). The term "pancreatic sufficiency" has been coined to describe the condition of the latter group of patients, who are generally older at diagnosis and have lower sweat chloride levels, milder respiratory disease, normal growth, and a better overall prognosis than patients with pancreatic insufficiency.[2,4] We have found, however, that in a small percentage of patients with pancreatic sufficiency but greatly reduced pancreatic function, pancreatic insufficiency develops with advancing age.[5-7]

Since there is a remarkable concordance of pancreatic-function status among affected members within the same family,[8] we have suggested that genetic factors could influence the degree of pancreatic disease and its rate of progression. In support of this hypothesis, a striking difference has been detected between patients with pancreatic sufficiency and those with insufficiency, with respect to allelic and haplotype distribution of DNA markers tightly linked to the locus of the cystic fibrosis gene.[9]

Through identification and isolation of the cystic fibrosis gene on chromosome 7,[10-12] we found that approximately 70 percent of the mutant chromosomes carried a specific 3-bp (base pair) deletion that results in the loss of a phenylalanine residue at amino acid position 508 of the putative gene product (ΔF_{508}). Data on the extended DNA-marker haplotype suggested that the remainder of the cystic fibrosis mutant gene pool consisted of multiple different mutations, and that patients with pancreatic sufficiency had mutant alleles that were different from those in patients with pancreatic insufficiency.[12] On the basis of these observations, we hypothesized that the nature of the mutations associated with cystic fibrosis might determine the phenotypic expression of the disease.[12]

In this report, we describe the results of an expanded study, in which many patients were evaluated, of the associations between clinical phenotypes of cystic fibrosis and the ΔF_{508} mutation. In addition to their pancreatic function, we examined clinical indexes such as pulmonary function, growth measurements, the presence or absence of meconium ileus, and sweat chloride levels.

METHODS

Patients

The study group consisted of 293 patients with cystic fibrosis who regularly attended the cystic fibrosis clinic at the Hospital for Sick Children, Toronto. The diagnosis had been previously confirmed in

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each patient by clinical findings of typical pulmonary disease or gastrointestinal disease[1] or a family history of cystic fibrosis (or any combination of these features), together with at least two abnormal values on sweat chloride tests. The initial diagnostic sweat chloride value was used to characterize the patients for this study. The study was performed with the approval of the Human Subjects Review Committee of the hospital, and consent was obtained from each patient or a parent or legal guardian. Since 1977, a broad range of clinical data on each patient has been recorded at the time of diagnosis and at each subsequent clinic visit (every three months) and incorporated into a computerized database.[13] The variables included in the analysis of clinical phenotypes included pancreatic function, pulmonary disease, growth and nutritional status, meconium ileus, and sweat chloride levels.

Pancreatic Function

Patients were characterized as having either sufficient or insufficient pancreatic function at the time of diagnosis. Subsequently, patients with pancreatic sufficiency were followed for evidence of pancreatic failure. Each patient's current pancreatic-function status was included in this analysis. The following previously described tests[12] were performed.

Fecal-Fat–Balance Studies

Stool samples were collected and pooled over a period of three to five days during which the patients' dietary fat intake was adequate for their ages. Dietary constituents were measured with the use of dietary scales. Stool fat content was analyzed according to the method of van der Kamer et al.,[14] and fat loss was expressed as a percentage of fat intake. In patients receiving medium-chain triglycerides in their diet, fecal analysis was performed according to the method of Jeejeebhoy et al.[15] Pancreatic function was considered insufficient if fecal fat loss exceeded 7 percent of dietary fat intake or, in infants less than six months of age, 15 percent of intake.[16] The fat-balance study was repeated in patients with pancreatic sufficiency if the development of pancreatic insufficiency was suspected — for example, if there was unexplained weight loss or an alteration in stool consistency.

Serum Cationic Trypsinogen

Random serum samples were obtained at intervals of 6 to 12 months and analyzed by a double-antibody radioimmunoassay technique.[17] The serum trypsinogen test reliably distinguished patients with pancreatic sufficiency from those with insufficiency after the patients reached seven years of age,[18] and it was used to monitor those with pancreatic sufficiency for evidence of progressive pancreatic disease.

Pancreatic-Stimulation Test

The pancreatic-stimulation test was performed in patients with pancreatic sufficiency in whom cystic fibrosis had been newly diagnosed or in whom deterioration of pancreatic function was suspected. Nasoduodenal intubation was performed with a double-lumen tube containing two ports; one port was positioned opposite the ampulla of Vater, through which a nonabsorbable marker was perfused at a constant rate, and the distal port was positioned at the ligament of Treitz for aspiration of mixed pancreatic secretions and the marker solution.[19] After an equilibration period, pancreatic secretions were collected for 1 hour (three 20-minute periods) during continuous intravenous administration of cholecystokinin and secretin. The loss of pancreatic juice from the distal duodenal collection port was expressed as a percentage of the volume of the infused marker that was recovered.[3] Pancreatic function was considered sufficient if the output of pancreatic colipase exceeded 120 units per kilogram of body weight per hour or if the trypsin output exceeded 50 units per kilogram per hour.[3,5]

Pulmonary Disease

Routine pulmonary-function tests were performed every six months in all patients over six years of age. Forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), and forced expiratory flow rate in the middle half of expiration (FEF_{25-75}) were measured and expressed as a percentage of predicted values for height and sex, according to previously described standardized pulmonary equations.[20] The results of each patient's most recent pulmonary-function test were used in this analysis.

Growth and Nutritional Status

Each patient's height and weight were recorded every three months at regular clinic visits. Height and weight percentiles and weight expressed as a percentage of the ideal weight for height were computed with use of the tables of Tanner et al.[21] Growth data from the most recent visit were used in this analysis.

Meconium Ileus

Meconium ileus was defined as a failure to pass meconium within 24 hours or more after birth, in conjunction with clinical signs of acute intestinal obstruction and characteristic radiologic findings.[22] The majority of patients with meconium ileus required surgical intervention.

Analysis of the ΔF_{508} Mutation

Total human genomic DNA samples were prepared from peripheral-blood samples. A region of the genomic DNA spanning the ΔF_{508} mutation was amplified by means of the polymerase chain reaction, and the amplification products were analyzed by hybridization to ^{32}P-labeled oligonucleotides specific for the mutant and the normal sequences, as described previously.[12] Each chromosome in the patient was characterized as carrying ΔF_{508} or another, as yet undefined ("other"), mutation; the genotype for each patient (i.e., $\Delta F_{508}/\Delta F_{508}$, ΔF_{508}/other, or other/other) was then confirmed with use of information derived from evaluation of the parents.

Statistical Analysis

Analysis of variance was used to assess the significance of differences among group means, with Bonferroni's adjustment of the P value for t-tests between group means. Chi-square analysis was used to compare differences among group proportions. Pulmonary function is known to decline with age, although the timing and rate of decline vary widely among individual patients. To account for the relation between pulmonary function and age and for significant differences in mean age among the groups studied, analysis of covariance was used to compare differences in pulmonary function among the groups.[23]

Results

We studied a total of 293 patients with cystic fibrosis who belonged to 233 families. To avoid sampling bias, only two parental chromosomes for cystic fibrosis were scored for each family when the prevalence of the ΔF_{508} mutation was computed. The overall prevalence of this mutation was 71 percent; 120 families (51 percent) had the ΔF_{508} mutation on both parental cystic fibrosis chromosomes, 90 (39 percent) on one, and 23 (10 percent) on neither. Of the patients, 52 percent were homozygous for ΔF_{508}, 40 percent were heterozygous for ΔF_{508}, and 8 percent carried other, undefined mutations on both chromosomes (i.e., the other/other genotype). The distribution of these mutations in the study population was that expected according to the Hardy–Weinberg law of equilibrium (chi-square = 0.49).

The diagnosis of cystic fibrosis was made in patients homozygous for ΔF_{508} at an earlier age than in those heterozygous for ΔF_{508} or those with the other/other genotype (Table 1). Pancreatic-function status was also strongly related to the presence of the ΔF_{508} mutation. Among the 151 patients who were homozygous

Table 1. Pancreatic-Function Status and Age at Diagnosis of 293 Patients with Cystic Fibrosis, According to Genotype (with Respect to the ΔF_{508} Mutation).

VARIABLE	$\Delta F_{508}/\Delta F_{508}$	ΔF_{508}/OTHER	OTHER/OTHER
No. of patients (% of cohort)	151 (52)	117 (40)	25 (8)
No. with pancreatic sufficiency at present (% of genotype group)*	2 (1)	33 (28)	16 (64)
Age at diagnosis — yr†	1.8±3.3	4.4±5.9	8.4±8.3

*P<0.001 by chi-square test (2 df).

†P<0.001 by analysis of variance of three means; values are means ±SD.

for ΔF_{508}, 1 percent (one patient 6 years old and another 15 years old) had pancreatic sufficiency, whereas 28 percent of those with the ΔF_{508}/other genotype and 64 percent of those with the other/other genotype had pancreatic sufficiency at the time of their most recent examination. Eight of the homozygous patients and three of the heterozygous patients had pancreatic sufficiency at diagnosis, but pancreatic insufficiency developed later in life (between 8 months and 21 years of age). If the mean age and distribution of the genotypes were assumed to be the same among the patients with pancreatic sufficiency and those with insufficiency, we would have expected at least 25 patients in the group that was homozygous for the ΔF_{508} mutation to have pancreatic sufficiency. There were no significant differences in sex distribution, sweat chloride values at diagnosis, current pulmonary function (FVC, FEV_1, or FEF_{25-75}), or most recent percentiles for weight and height in the three genotype groups.

When the patients in the three genotype groups were divided according to their current pancreatic-function status (Table 2), it became apparent that the differences between these groups in the average age at diagnosis (Table 1) were directly related to differences in the proportions with pancreatic sufficiency. Although patients with pancreatic insufficiency in the three groups did not differ significantly in age at diagnosis or current age, they were significantly younger (both at diagnosis and in current age) than those with pancreatic sufficiency who were in the ΔF_{508}/other and other/other groups. The mean sweat chloride values at diagnosis were also significantly lower in the patients with pancreatic sufficiency who had the ΔF_{508}/other and other/other genotypes.

Meconium ileus, which was a presenting complication in 36 patients (12 percent), occurred only in those with pancreatic insufficiency but was not associated with any one of the three genotypes. The growth characteristics of the patients also differed according to pancreatic-function status. The percentiles for current weight of the patients with pancreatic insufficiency were significantly lower than those of the patients with pancreatic sufficiency (except for two patients with pancreatic sufficiency who were homozygous for ΔF_{508}). When current weight was measured as a percentage of ideal weight for height, there was also a significant difference between patients with pancreatic insufficiency and those with pancreatic sufficiency; values in the group with pancreatic insufficiency were near normal, and those in the group with pancreatic sufficiency were above normal. There was, however, no significant difference in height expressed as a percentile. The two patients with pancreatic sufficiency who were homozygous for ΔF_{508} had clinical characteristics at diagnosis (age at diagnosis and sweat chloride concentrations) and at the time of their most recent evaluation (growth percentiles, pulmonary function, and current age) that were similar to those of the patients with pancreatic insufficiency who had the same genotype.

Possible relations between genotype and pulmonary function were examined next. Three measurements (FVC, FEV_1, and FEF_{25-75}) were used to assess pulmonary status (Table 3). The results in the 241 patients who were old enough to permit pulmonary-function testing were included in the analysis. Because of the strong dependence of pulmonary function on age, the tests for differences among the three genotype groups and between the pancreatic-function groups (patients with pancreatic sufficiency and those with insufficiency) were not straightforward. Least-squares regression lines (pulmonary-function variable vs. age) were computed and tested for significant deviation from a common slope ($P = 0.27$, 0.38, and 0.66 for FVC, FEV_1, and FEF_{25-75}, respectively). Then, for each pulmonary-function variable, regression lines were fitted with a common slope for all groups. This method allowed the estimation and comparison of mean values for each group at any given age. The result of this analysis, as shown in Table 3, revealed a significant difference between the patients with pancreatic insufficiency and those with pancreatic sufficiency; the F test for differences among the adjusted group means demonstrated significant differences in FEV_1 ($P = 0.001$), FVC ($P = 0.017$), and FEF_{25-75} ($P = 0.001$). The individual group comparisons demonstrated that the patients

Table 2. Clinical Characteristics of the 293 Patients, According to Genotype and Pancreatic-Function Status.*

CHARACTERISTIC	$\Delta F_{508}/\Delta F_{508}$		ΔF_{508}/OTHER		OTHER/OTHER	
	PI	PS	PI	PS	PI	PS
No. of patients	149	2	84	33	9	16
No. with meconium ileus (%)	22 (15)	0	10 (12)	0	4 (44)	0
Age at diagnosis — yr†	1.8±3.3	0.6±0.1	2.8±4.1‡	8.4±7.6	4.2±10.2‡	11.0±6.0
Sweat chloride level at diagnosis — mmol/liter†	106±16	112±15	108±18‡	94±18	115±15‡	89±15
Current age — yr†	17±10	10±6	18±11‡	26±9	11±11‡	23±9
Current weight percentile†	39±30	32±2	42±31‡	64±29	31±31‡	73±19
Current height percentile	40±28	73±12	45±28	50±26	44±35	55±21
Current weight for height — %†	100±12	86±2	98±14‡	108±15	95±8‡	110±15

*PI denotes pancreatic insufficiency, and PS pancreatic sufficiency. Plus–minus values are means ±SD.

†P<0.001 by analysis of variance of five means (group in which n = 2 was not included in analysis).

‡Significantly different from the value for patients with pancreatic sufficiency within each genotype group (P<0.05, with Bonferroni's correction).

Table 3. Current Pulmonary Function in 241 Patients Old Enough for Testing.*

VARIABLE	$\Delta F_{508}/\Delta F_{508}$		ΔF_{508}/OTHER			OTHER/OTHER			P VALUE†
	PI	PS	PI		PS	PI		PS	
No. of patients	121	1	66		32	6		15	—
FEV_1 — % predicted	61	68	68	(0.01)	81	60	(0.10)	80	0.001
FVC — % predicted	72	72	76	(0.02)	88	66	(0.13)	83	0.017
FEF_{25-75} — % predicted	42	53	50	(0.02)	65	40	(0.05)	68	0.001

*Values are means adjusted to the overall mean age (20 years) computed from regression equations with a common slope for each pulmonary variable. Values in parentheses are P values for the comparison of means within genotype groups.

†By F test for differences among mean values in analysis of covariance.

with pancreatic sufficiency had consistently better pulmonary function than those with pancreatic insufficiency. The single patient with pancreatic sufficiency who was homozygous for ΔF_{508} and underwent pulmonary-function testing had values similar to those of the patients with pancreatic insufficiency who had this genotype. There were too few patients in the other/other genotype group (6 patients with pancreatic insufficiency and 15 with pancreatic sufficiency) to allow definitive comparison; however, the P values and the actual pulmonary-function values were consistent with those in the ΔF_{508}/other group. Figure 1 shows data on FEV_1 for individual patients in the genotype groups, together with the regression lines that were used to compute the adjusted mean values for pulmonary function. The variability in pulmonary function was considerable at all ages, but the clear separation of the regression lines for patients with pancreatic sufficiency in two genotype groups (ΔF_{508}/other and other/other) demonstrated the consistent differences between these groups.

Twenty-one of the 293 patients have died, at ages ranging from 1 month to 27 years. They were distributed proportionately among the three genotype groups, and all had pancreatic insufficiency. The pulmonary-function and anthropometric data shown in Tables 2 and 3 and Figure 1 include measurements made at these patients' last regular clinic visits. Although their values were generally in the lower ranges, reanalysis of the data without the values for these 21 patients produced similar results. Some P values were altered, but all significant differences remained significant.

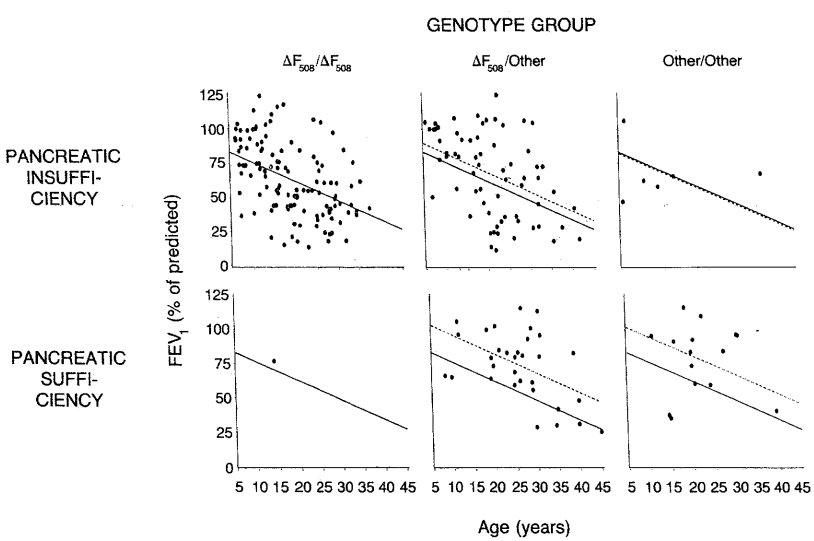

Figure 1. Forced Expiratory Volume in One Second (FEV_1) Plotted against Age in Individual Patients, According to Genotype Group and Pancreatic-Function Status.

The least-squares regression lines are constrained to have a common slope since the slopes of separately fitted lines were not significantly different (P = 0.32). The regression lines for the patients with pancreatic insufficiency (top panels) were similar to each other. The regression lines for the patients with pancreatic sufficiency who were in the ΔF_{508}/other and other/other genotype groups (bottom panels) also were similar to each other, but they were significantly higher than the regression line for the patients with pancreatic insufficiency in the $\Delta F_{508}/\Delta F_{508}$ genotype group (P = 0.003).

The solid line denotes the regression line for the patients with pancreatic insufficiency who had the $\Delta F_{508}/\Delta F_{508}$ genotype; this line was used as the reference for all groups. The dashed line denotes the regression line for the group in each panel (except the single patient with pancreatic sufficiency in the $\Delta F_{508}/\Delta F_{508}$ genotype group).

DISCUSSION

The identification of the cystic fibrosis gene has provided a focus for studies attempting to elucidate the basic defect and pathophysiology of the disease. This study is our first major attempt to understand the varied symptoms in cystic fibrosis through analysis of the genotypes of patients with respect to ΔF_{508}, the principal mutation that causes the disease. We found that the extreme variability in disease severity is partly reflected by the different genotypes for cystic fibrosis ($\Delta F_{508}/\Delta F_{508}$, ΔF_{508}/other, and other/other). We previously reported a significant association between genotype and pancreatic-function status[12] — an association confirmed here in a larger population. The vast majority (99 percent) of patients homozygous for the ΔF_{508} mutation had pancreatic insufficiency at diagnosis or later, whereas the other/other genotype group had the fewest patients with pancreatic insufficiency. When the patients in each genotype group were divided according to their pancreatic-function status, a consistent difference in disease severity among the three genotypes could be detected. In all three genotype groups the patients with pancreatic insufficiency tended to have more severe disease, as reflected by an earlier age at diagnosis, higher sweat chloride concentrations at the time of diagnosis, worse pulmonary disease, and lower percentiles for weight. In contrast, the patients with pancreatic sufficiency who carried one copy of ΔF_{508} or none had milder

disease that was generally recognized at an older age with significantly lower diagnostic sweat chloride concentrations; they had better pulmonary function after adjustment for age and nutritional status at all ages.

It is unclear whether the more severe symptoms associated with pancreatic insufficiency could be due to general malnutrition in these patients, direct consequences of the basic defect, or both. The fact that patients with pancreatic sufficiency tended to have lower values for the sweat chloride level — a variable unlikely to be related to nutritional status — argues for the latter possibility, as does the remarkable concordance of pancreatic-function status within families that we have previously described. Furthermore, the values for weight as a percentage of ideal weight for height (Table 2) indicate that most of our patients were not undernourished. Thus, patients with pancreatic sufficiency who had the ΔF_{508}/other and other/other genotypes appear to have a mutant cystic fibrosis gene product that confers a mild disease phenotype, with pancreatic sufficiency as one of the consequences. Since the ΔF_{508} mutation is associated with a more severe phenotype, the mild phenotype must be conferred by "other" cystic fibrosis alleles in the patients with pancreatic sufficiency. We have previously proposed that mutant alleles be classified as "mild" or "severe"[12] (i.e., producing mild or severe disease), with the mild allele having a dominant effect over the severe allele; our current, expanded data provide additional support for this hypothesis.

Pancreatic insufficiency develops in a small percentage of patients with pancreatic sufficiency but reduced pancreatic function.[6,7] A recent report by Waters et al.[24] revealed that 38 percent of patients in whom cystic fibrosis was diagnosed by neonatal screening had pancreatic sufficiency. Although this number is much higher than the proportion of patients with pancreatic sufficiency in our cross-sectional study, pancreatic failure developed by three years of age in approximately 20 percent of the patients who had had pancreatic sufficiency,[24] again indicating the possibility that pancreatic insufficiency develops with age in some patients. Our previous pancreatic-stimulation studies[7] suggested that patients with pancreatic sufficiency who are at risk of pancreatic failure have greatly reduced secretion of pancreatic fluid and enzymes at an early age, whereas those who retain pancreatic sufficiency over an extended period have levels of enzyme secretion closer to the control range. In our study, the majority of patients with pancreatic sufficiency who were homozygous for the ΔF_{508} deletion subsequently had pancreatic insufficiency, which suggests that their exocrine pancreatic function was severely compromised. Since we expect that in time pancreatic failure will occur in the two remaining patients with pancreatic sufficiency who were homozygous for the mutation, all such patients should be closely monitored for evidence of impending pancreatic failure. Furthermore, the three patients with pancreatic sufficiency and the ΔF_{508}/other genotype in whom pancreatic insufficiency subsequently developed are likely to carry a severe mutation on their "other" cystic fibrosis chromosome.

Our cohort has not been followed long enough to permit meaningful comparison of survival in the three genotype groups. Thus far, only 21 patients have died, all of whom were patients with pancreatic insufficiency, distributed among the three groups. Since survival is directly correlated with the severity of pulmonary disease, we predict that there will be significant differences in survival between patient groups with mild and severe phenotypes. The expression of other, less common clinical manifestations of cystic fibrosis, including liver dysfunction, diabetes mellitus, and the distal intestinal obstruction syndrome, could also be correlated with specific mutations within the cystic fibrosis gene, but such correlations await definition of the remaining mutations and a complete genetic analysis of our entire clinic population of approximately 530 patients.

The overall proportion of patients with the ΔF_{508} allele in the families studied was 0.71 — not significantly different from the frequencies of 0.68 and 0.75 reported in previous studies.[12,25] Our preliminary (unpublished) data indicate that the remaining cystic fibrosis mutations are rather heterogeneous. As a result, features showing an association between genotype and phenotype — other than pancreatic function — may not be as obvious, especially clinical features not shared by affected members within the same family. For example, the prevalence of meconium ileus was about 12 percent in our cohort, and there is only 30 percent concordance of this clinical symptom within families.[26] Although meconium ileus was associated with all three genotype groups (ΔF_{508}/ΔF_{508}, ΔF_{508}/other, and other/other), it was found exclusively in patients with pancreatic insufficiency but was not mandatorily expressed in all of them. Thus, meconium ileus appears to be part of the phenotype of severe cystic fibrosis, a finding consistent with our previous hypothesis[26] that other genetic or environmental factors may be required for the development of this manifestation.

Notwithstanding the possibility that additional factors may contribute to the severity of the disease, our data suggest that there is a correlation between genotype and phenotype in cystic fibrosis. The most striking example of this is the association between pancreatic status and the presence of the ΔF_{508} mutation. As additional mutations accounting for the remaining cystic fibrosis chromosomes are identified, our observation will be expanded to include other mutations and clinical features. Furthermore, the variability of pulmonary function in all the study groups indicates that additional factors affect the severity or progression of lung disease. Therefore, information on morbidity and prognosis should be used with caution in counseling families seeking prenatal diagnosis or heterozygote detection in cystic fibrosis.[25] Parallel studies in mutational analysis and clinical investigation should also provide important insights into the patho-

physiology and basic defect of this disease. To detect any further correlations, however, larger patient populations will be required, because the prevalence of each of the remaining mutations is likely to be low and to vary with geographic location.

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