Predictors of Deterioration of Lung Function in Cystic Fibrosis

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Summary. The severity of lung disease in cystic fibrosis (CF) may be related to the type of mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, and to environmental and immunological factors. Since pulmonary disease is the main determinant of morbidity and mortality in CF, it is important to identify factors that can explain and predict this variation. The aim of this longitudinal study of the whole Swedish CF population over age 7 years was to correlate genetic and clinical data with the rate of decline in pulmonary function. The statistical analysis was performed using the mixed model regression method, supplemented with calculation of relative risks for severe lung disease in age cohorts.

The severity of pulmonary disease was to some extent predicted by CFTR genotype. Furthermore, the present investigation is the first long-term study showing a significantly more rapid deterioration of lung function in patients with concomitant diabetes mellitus. Besides diabetes mellitus, pancreatic insufficiency and chronic *Pseudomonas* colonization were found to be negative predictors of pulmonary function. In contrast to several other reports, we found no significant differences in lung function between genders. Patients with pancreatic sufficiency have no or only a slight decline of lung function with age once treatment is started, but an early diagnosis in this group is desirable. **Pediatr Pulmonol. 2002; 33:483–491.** © 2002 Wiley-Liss, Inc.

Key words: cystic fibrosis; CFTR mutation; mixed model regression model; pulmonary disease; pulmonary function; pancreatic function; *Pseudomonas* colonization; diabetes mellitus.

INTRODUCTION

The discovery of the cystic fibrosis transmembrane conductance regulator (CFTR) gene in 1989[1] and the identification of multiple mutations raised the question of a possible relationship between genotype and phenotype. The expectations that the type of CFTR mutation would predict the clinical course and would be useful for individualizing treatment have only partly been fulfilled. There is a wide variation in disease severity even among patients with the same mutations. An association has been found between CFTR genotype and pancreatic function,[2] although some mutations cannot be classified as either mild or severe with regard to pancreatic function.[3,4] Furthermore, in some patients, pancreatic insufficiency develops only with increasing age.[5] Mutations associated with pancreatic sufficiency either result in a CFTR protein with some residual activity,[6,7] or a reduced amount of normal messenger RNA (mRNA).[8,9]

Whether pulmonary function is related to the CFTR genotype is less clear. Some investigators have indicated such an effect,[10,11] while others have not.[12,13] Only a few mutations have so far been associated with mild lung involvement.[7,14] The fact that severity of lung disease varies in patients with the same CFTR genotype suggests that other genetic, environmental, or immunological factors, as well as bacterial colonization and differences

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in treatment, are important. Since progressive obstructive lung disease is the main determinant of morbidity and mortality in cystic fibrosis (CF), it is essential to identify factors that can explain and predict variability. A fairly new statistical approach, mixed model regression analysis, has proven valuable in estimating average rates of decline in pulmonary function, and can incorporate the effects of various clinical characteristics.[15,16]

We report on a study of all patients over 7 years of age in the CF population of Sweden. The aim of the study was to identify risk factors for progression of lung disease in CF. Genetic and clinical data were correlated with rate of deterioration of forced expiratory volume in 1 sec (FEV_1) and vital capacity (VC), using mainly mixed model regression analysis.

PATIENTS AND METHODS

Patients

The total CF population in Sweden comprised 475 patients in December 31, 1998. Data from 343 living patients attending the CF centers in Stockholm, Gothenburg, Uppsala, and Lund, and born before January 1, 1993, were collected. Thirty-four deceased patients (25 deceased during the 1990s and 9 during the 1980s) were also included in the analysis. Twenty-one patients, of whom 16 were still alive at time of this study, had been lung-transplanted. To be included in the study, patients had to have done at least two lung function tests. The rates of decline in FEV_1 and VC were analyzed in relation to the following clinical characteristics: genotype, gender, pancreatic function, chronic *Pseudomonas* colonization, diabetes mellitus, and liver disease.

The diagnosis of CF was confirmed by typical clinical findings and abnormal sweat chloride concentrations (>60 mmol/L for children and >80 mmol/L for adults), measured by the quantitative pilocarpin iontophoresis method.

ABBREVIATIONS	
ANOVA	Analysis of variance
BMI	Body mass index
CL	95% confidence limits
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
DNA	Deoxyribonucleic acid
FEV_1	Forced expiratory volume in 1 sec
FVC	Forced vital capacity
LSD	Least significant difference
mRNA	Messenger ribonucleic acid
PA	Chronic *Pseudomonas aeruginosa* colonization
PI	Pancreatic insufficiency
PS	Pancreatic sufficiency
RR	Relative risk
SD	Standard deviation
VC	Vital capacity
WHO	World Health Organization

Clinical characteristics were extracted from the records and from the Swedish computerized CF database. The CFTR gene was analyzed by established DNA molecular techniques. Patients were subdivided into four genotype groups: A) homozygous ΔF508/ΔF508; B) severe/severe mutation (including ΔF508 in heterozygous form, and in nonsense and frame-shift mutations); C) missense/severe mutation or missense/missense mutation; and D) one or two unknown mutations.

Patients were classified as either pancreatic sufficient (PS) or insufficient (PI) by one or more of the following criteria: abnormal pancreatic stimulation test, low fecal elastase concentration (< 200 µg elastase 1 (E1)/g), and low chymotrypsin activity (< 0.22 mkat/kg).

Chronic *Pseudomonas aeruginosa* colonization (PA) was defined as three positive consecutive sputum cultures over a period of 6 months, and/or by a significant increase in specific serum anti-*Pseudomonas* precipitins,[17] and/or by *Pseudomonas* anti-exotoxins.[18]

Respiratory status was assessed by tests of VC and FEV_1, expressed as percentages of predicted values for height, weight, age, and gender. To guarantee reliable spirometric measurements, we used those regularly performed (once or twice a year) at the Departments of Clinical Physiology. All measurements were done when the patients were in optimal clinical condition. The total number of spirometric tests used in the analysis was 3,441 and ranged from 2–28 in individual patients, with a mean of 9 and a median of 8. The duration of follow-up ranged from 0.5–27 years, with a mean of 10 years and a median of 8.5 years. Mean age at first lung function test was 11 years, and the median age 7.5 years.

From age 10 years, patients were routinely screened every second or third year with an oral glucose tolerance test. The latest result was used for the present study. In lung-transplanted patients, the latest result before the operation was used. Diabetes mellitus was defined according to World Health Organization (WHO) criteria.[19]

Liver cirrhosis was diagnosed on the basis of irregular echogenicity of the liver parenchyma at ultrasound scanning and/or on biopsy. Biochemical liver involvement was defined as persistent abnormal values of liver enzymes (aspartate aminotransferase > 0.7 µkat/L, alanine aminotransferase > 0.7 µkat/L, and glutamyl-transferase > 1.2 µkat/L) for at least 2 consecutive years.

Statistical Analyses

Statistical calculations were made using SAS (version 6.12 for Windows). PROC MIXED in SAS was used when analyzing the decline of FEV_1 and VC over time, to obtain a mixed regression model of data. The general mixed model equation was described by Laird and Ware in 1982.[20] The advantage of the mixed model is that random errors both within and between individual

patients are considered in one model. The rates of decline of FEV_1 and VC vs. age were calculated with genotype, gender, pancreatic status, fatal outcome/lung transplantation, and chronic *Pseudomonas aeruginosa* infection as covariates in separate models, and for patients with pancreatic insufficiency (PI), with diabetes and liver disease also as covariates. The intercept was set at 5 years, because from this age on, reliable measurements of pulmonary function can be obtained. Intercept and age at observation were considered as random effects regressors, and the covariates described above were used as fixed effects.

Annual rate of deterioration of FEV_1 in PI patients with or without diabetes was further investigated in two subanalyses. In the first subanalysis, all PI patients were included, but only FEV_1 values recorded before age 15 years were used in an attempt to find out whether patients who developed diabetes had a faster decline in FEV_1 early in life and before showing signs of diabetes. In the second analysis, only PI patients born before January 1, 1984 were included, and all their FEV_1 data were used. The latter analysis was done to eliminate the influence of patients under age 15, whose future fate with regard to diabetes is unknown.

In addition to the mixed model regression analysis, we also calculated the relative risk (RR) of having a severely reduced FEV_1, defined as $< 60\%$, with regard to the variables mentioned above.

The longitudinal FEV_1 data were grouped into four cohorts: data obtained at a patient age of 1) up to 12 years, 2) between 13–18, 3) between 19–24, and 4) between 25–30 years. Depending on the age of the patient, his or her FEV_1 could be included in more than one cohort, but only the latest FEV_1 value obtained within each age interval was used.

The tests comparing genotypes in Table 2 were performed in a two-stage procedure to avoid a problem with multiple testing and mass significance. 1) First, for *PA, diabetes, and cirrhosis*, overall chi-square tests for each of the three 2 by 4 (A, B, C, and D) cross-tables were performed. If a *P*-value was above 0.05, no further tests were done. 2) If a *P*-value was below 0.05, pairwise tests (chi-square or Fisher's exact test) between all four genotypes were performed. *Mean age at diagnosis* was first tested with one-way ANOVA, followed by pairwise comparisons using least significance difference (LSD) in the ANOVA.

Comparisons of continuous variables were made with *t*-test or Mann-Whitney test.

RESULTS

The frequencies of the 10 most common CFTR mutations found in the Swedish CF population are shown in Table 1, and clinical characteristics in Table 2. The

TABLE 1—Allele Frequencies of 10 Most Common CFTR Mutations in Swedish CF Population

Mutation	Allele frequency (%)
ΔF508	67.9
394delTT	7.1
3659delC	6.4
S945L	1.2
R117C	1.0
R117H	0.55
T338I	0.55
G551D	0.55
R553X	0.55
I506L	0.41

mean age of all patients was 21.9 years. The proportions of patients with pancreatic sufficiency, diabetes mellitus, and liver cirrhosis were 14.1%, 15.4%, and 5.6%, respectively. Cirrhosis was always associated with pancreatic insufficiency, but biochemical liver abnormalities were found also in PS patients. Diabetes mellitus was found exclusively in PI patients. There was no gender predominance among patients with pancreatic sufficiency, diabetes mellitus, or cirrhosis. Age at diagnosis was significantly higher, and *Pseudomonas* colonization was significantly lower in patients with missense mutations and in patients with pancreatic sufficiency. The CFTR mutations in patients with pancreatic sufficiency are listed in Table 3.

In patients who were deceased or had been lung-transplanted, chronic *Pseudomonas* colonization was significantly more frequent than in other patients (92% vs. 47%, $P = 0.001$).

The decline in FEV_1 with age was analyzed by mixed model regression, with genotype, gender, pancreatic status, and fatal outcome/lung transplantation as covariates. The results are shown in Table 4, and represented graphically in Figures 1–4. Figure 1 demonstrates a slower rate of decline of FEV_1 in patients with missense mutations (group C) compared with the other genotypes ($P = 0.01$ when compared with ΔF508/ΔF508; Table 4). In group C, the decline was not different from zero. Groups A, B, and D did not differ from each other. Figure 2 shows a comparison between males and females. An apparent difference between genders was not statistically significant ($P = 0.16$, Table 4).

Figure 3 shows that the slope indicating deterioration in FEV_1 in patients who were deceased or had been lung-transplanted was steeper than for those still alive and not transplanted ($P = 0.0001$). The same was true for VC ($P = 0.0001$). The annual declines of FEV_1 and VC in the former group (2.0%/year and 1.6%/year, respectively) were the highest found for all patient groups (Table 4). Furthermore, the inferred values for FEV_1 and VC at age 5 years (the intercepts) were significantly lower

TABLE 2—Clinical Characteristics of Swedish Study Population[1]

No. of pats. (%)	No. of pats. with PA (%)	No. of pats. with diabetes (%)	No. of pats. with cirrhosis (%)	Mean age at diagnosis (yr)
Total, 377 (100)	197 (52.3)	58 (15.4)	21 (5.6)	3.7 ± 7.2
Female, 191 (50.7)	107 (56)	29 (15.2)	12 (6.3)	
Male, 186 (49.3)	90 (48.4)	29 (15.6)	9 (4.8)	
Genotypes				
Group A 167 (44.3)	88 (52.7)[2]	31 (18.6)[3]	13 (7.8)[4]	2.1 ± 4[5]
Group B 100 (26.5)	57 (57)	16 (16)	6 (6)	1.8 ± 2.5
Group C 48 (12.7)	15 (31.3)	2 (4.2)	0 (0)	13.5 ± 13
Group D 46 (12.2)	25 (54.3)	7 (15.2)	2 (4.3)	5.3 ± 9.6
NA 16 (4.2)	12 (75)	2 (12.5)	0 (0)	
Pancreatic function				
PS 53 (14.1)	12 (22.6)*	0 (0)*	0 (0)	13.7 ± 13*
PI 324 (85.9)	185 (57.1)	58 (17.9)	21 (6.5)	2.1 ± 3.8

[1]Frequencies of chronic *Pseudomonas aeruginosa* infection (PA), diabetes, and liver cirrhosis are given for all patients, for patients with variable genotypes, and for patients with pancreatic sufficiency (PS) and pancreatic insufficiency (PI), respectively. Mean ages ($\pm$ SD) at diagnosis in various groups are also given. Genotype C was associated with significantly lower frequency of PA compared with groups A, B, and D. Age at diagnosis was significantly higher in group C than in the other genotype groups (A, B, and D). Pancreatic sufficiency was associated with significantly lower frequency of PA and diabetes mellitus, and a higher age at diagnosis than pancreatic insufficiency. NS, not significant; NA, not analysed; Genotypes; A, homozygosity for ΔF508; B, severe/severe mutation; C, one or two missense mutations; D, one or two unknown mutations. pats., patients; yr, years.
[2]$P = 0.025$ for overall chi-square test of groups A, B, C, and D. A vs. C, $P = 0.009$; B vs. C, $P = 0.003$; D vs. C, $P = 0.024$. Other comparisons, NS.
[3]$P = 0.115$ (NS) for overall chi-square test of groups A, B, C, and D.
[4]$P = 0.228$ (NS) for overall chi-square test of groups A, B, C, and D.
[5]$P < 0.001$ tested with one-way ANOVA. Pairwise comparison using LSD in the ANOVA showed that group C differed significantly from A, B, and D.
*$P < 0.05$.

compared with those in the other CF patients (63.4% and 68.2% vs. 89% and 93.3%).

Figure 4 gives a comparison between patients with pancreatic sufficiency and insufficiency. PS patients had an annual deterioration of FEV_1 (0.187%/year) that was not significantly different from zero ($P = 0.48$) and clearly different from that of PI patients ($P = 0.01$, Table 4). The difference between the two groups was large, as shown in Figure 4.

The influence of chronic *Pseudomonas aeruginosa* infection (PA) is shown in Table 5 and Figure 5. PS patients had a lower rate of colonization (Table 2), and so PS and PI patients were analyzed separately. PI patients with PA had a significantly faster decline of FEV_1 than PI patients without PA ($P = 0.03$). The difference between

TABLE 3—CFTR Mutations Associated With Pancreatic Sufficiency in Swedish CF Population

Y109C	S549I/S549I
Y109N	S945L
R117C	N1088D $-$ R75Q
R117H	G1244E
L206W	$711 + 3A \rightarrow G$
T338I	$1249 - 5A \rightarrow G$
A455E	$2789 + 5G \rightarrow A$
I506L	5T

these groups was large, as demonstrated in Figure 5. PS patients with PA did not differ from PS patients without PA with regard to slope of the regression line ($P = 0.83$). The intercept on the Y axis, however, was significantly lower for PS patients with PA than for those without (75.5% vs. 93.2%; $P = 0.02$). When PS and PI patients without PA were compared, a possible difference did not reach statistical significance ($P = 0.23$). This may be misleading, however, as the decline in FEV_1 for PI patients without PA was significantly different from zero ($P = 0.0006$), but that for PS patients was not ($P = 0.53$).

PS patients had neither diabetes nor liver cirrhosis; the effects of these variables on FEV_1 and VC were studied only in PI patients. Table 6 shows the results for FEV_1. Figure 6 demonstrates that patients with diabetes mellitus had a faster decline in FEV_1 than patients without ($P = 0.02$). When we analyzed only those FEV_1 data that had been recorded up to age 15 years, no difference was found between those patients who later developed diabetes and those who did not ($P = 0.83$). When we analyzed only patients over age 15 years, those with diabetes had a significantly steeper decline of FEV_1 ($P = 0.01$), similar to those of all CF patients with diabetes. No difference was found between patients with and without liver cirrhosis ($P = 0.84$, Table 6).

TABLE 4—Mixed Model Regression Analysis of Rate of Decline in FEV_1 vs. Age[1]

		P-values for differences to the reference groups	
	Mixed model equation	Intercept	Slope
Genotype			
Group A (ref. gr)	$Y = 88.7 - 0.790 \times (age - 5)$		
Group B	$Y = 85.9 - 0.889 \times (age - 5)$	$P = 0.29$	$P = 0.65$
Group C	$Y = 85.8 - 0.043 \times (age - 5)$	$P = 0.5$	$P = 0.01*$
Group D	$Y = 82.8 - 0.820 \times (age - 5)$	$P = 0.12$	$P = 0.92$
Gender			
Male (ref. gr)	$Y = 86.2 - 0.644 \times (age - 5)$		
Female	$Y = 85.0 - 0.912 \times (age - 5)$	$P = 0.63$	$P = 0.16$
Pancreatic status			
PS (ref. gr)	$Y = 88.9 - 0.187 \times (age - 5)$		
PI	$Y = 85.1 - 0.891 \times (age - 5)$	$P = 0.36$	$P = 0.01*$
Fatal outcome/lung transplantation			
Dead/lung tr. (ref. gr)	$Y = 63.4 - 2.04 \times (age - 5)$		
Alive, no lung tr.	$Y = 89 - 0.567 \times (age - 5)$	$P = 0.0001*$	$P = 0.0001*$

[1]FEV_1 was expressed as percentage of predicted value for height, weight, age, and gender. Genotype, gender, pancreatic status, and fatal outcome/lung transplantation were covariates. Mixed model equation is here expressed as $Y_{age} = Y5 - k \times (age - 5)$, where intercept Y_5 is estimated value of FEV_1 at age 5, and slope k is annual decline of FEV_1. For each covariate, one subgroup was chosen as reference group (ref. gr) with which the other subgroup or subgroups were compared. Genotype A, homozygosity for ΔF508; B, severe/severe mutation; C, one or two missense mutations; D, one or two unknown mutations. PS, pancreatic sufficiency; PI, pancreatic insufficiency. tr., transplant.
*Statistical significance.

Except for the group of patients who had died or had been lung-transplanted, no significant differences in deterioration of VC were found between the various subgroups (data not shown). This fact further demonstrates that FEV_1 is the variable of lung function that best reflects the progression of lung disease in CF,[15] and impaired VC is seen only in late stages of the disease.
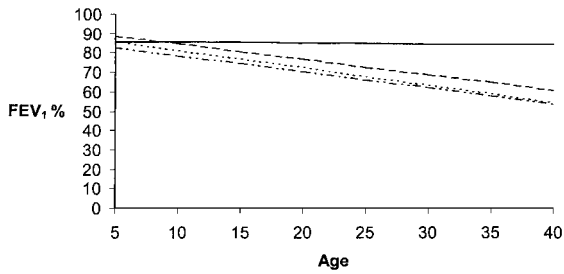

Fig. 1. Mixed model regression lines of FEV_1 (as % of predicted values) vs. age in years for four genotype groups: A, homozygosity for ΔF508; B, severe/severe mutation; C, one or two missense mutations; and D, one or two unknown mutations.
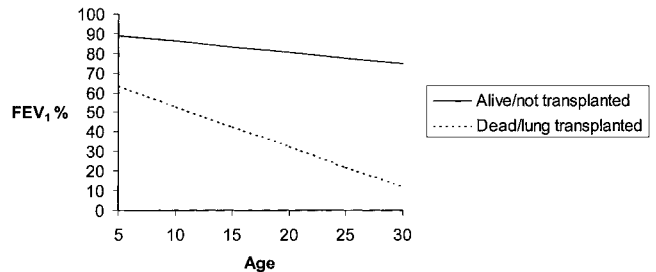

Fig. 3. Mixed model regression lines of FEV_1 vs. age in years for dead/lung-transplanted patients and living, nontransplanted patients.
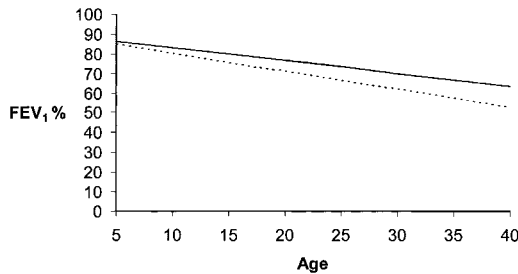

Fig. 2. Mixed model regression lines of FEV_1 vs. age in years for male and female patients.
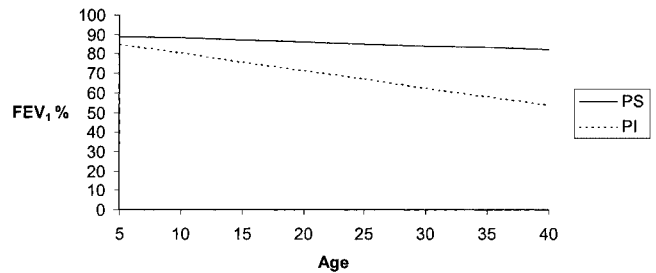

Fig. 4. Mixed model regression lines of FEV_1 vs. age in years for patients with pancreatic sufficiency (PS) and with pancreatic insufficiency (PI).

TABLE 5— Mixed Model Regression Analysis of Rate of Decline in FEV$_1$ vs. Age in Patients With Pancreatic Insufficiency and Pancreatic Sufficiency[1]

	Mixed model equation	P-values for differences to the reference groups	
		Intercept	Slope
PI + PA (ref. gr)	Y = 78.8 − 1.02 × (age − 5)		
PI + Non-PA	Y = 94.4 − 0.526 × (age − 5)	P = 0.0001*	P = 0.03*
PS + PA (ref. gr)	Y = 75.3 − 0.316 × (age − 5)		
PS + Non-PA	Y = 93.2 − 0.226 × (age − 5)	P = 0.03*	P = 0.83*

[1]Chronic *Pseudomonas aeruginosa* infection (PA) was the covariate, and comparison between subgroups was as detailed in Table 4. ref. gr, reference group.
*Statistical significance.

To further corroborate our conclusions, we calculated the relative risks (RR) of having a severely reduced FEV$_1$ with regard to the same variables as in the mixed model regression analysis. The endpoint for FEV$_1$ was set at 60% of predicted values, as values below 60% manifest clinically severe disease. The FEV$_1$ data were divided into age cohorts, as described in Patients and Methods. The results are shown in Table 7 and conform, in general, to those obtained in the mixed model regression analysis. The increased risk for patients with chronic *Pseudomonas aeruginosa* infection was seen at all ages, and the increased risk for patients with diabetes was noted in the oldest cohorts. An effect of genotype was seen only in the 19–24-year cohort due to the small number of group C patients in the other cohorts. Likewise, the effect of pancreatic status was not seen in the oldest cohort, which contained only a few patients.

DISCUSSION

A relationship between CFTR genotype and severity of pulmonary disease in CF was difficult to establish in several prior studies.[10–13] Most of these were based on cross-sectional data. A longitudinal investigation of pulmonary function is more informative, and the mixed model regression approach has proven to give a better statistical assessment in this context.[15]

Corey et al. in 1997,[15] using this method, showed that pancreatic insufficiency was associated with worse
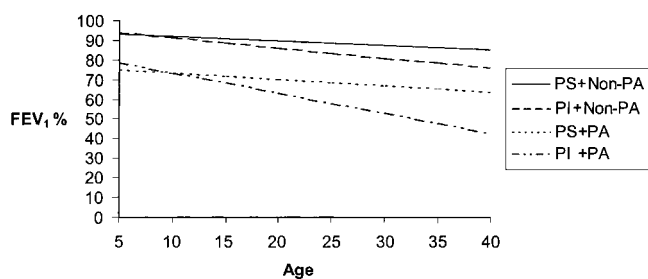

Fig. 5. Mixed model regression lines of FEV$_1$ vs. age in years for PI and PS patients with chronic *Pseudomonas aeruginosa* colonization (PA) and those without (Non-PA).

outcome of CF lung disease. In agreement with this study, we found a lower rate of decline in FEV$_1$ in patients with pancreatic sufficiency, which was not statistically different from zero. The difference between the PI and PS groups was impressive, as seen in Figure 4. The conclusion is that many PS patients can be expected to have mild pulmonary disease in the long term, unlike most PI patients.

A significantly slower deterioration of FEV$_1$ was found in patients with missense mutations compared with patients homozygous for ΔF508. Among the many CFTR missense mutations on record (Cystic Fibrosis Consortium), some confer a pancreatic-sufficient phenotype,[7] while others show a phenotype indistinguishable from that in patients homozygous for ΔF508.[7,21] Among our patients with missense mutations, a large portion (78%) was pancreatic-sufficient, a fact that probably contributed to the milder lung disease in this group. The data indicate that CFTR genotypes associated with long-term pancreatic sufficiency have more benign lung disease and better pulmonary function.

In contrast to Corey et al.[15] and other reports,[16] we found no significant difference in lung function between genders. The intensive and aggressive treatment used in CF centers in Sweden may obscure the previously demonstrated female vulnerability.

In accordance with Corey et al.,[15] we found the steepest annual decline of FEV$_1$ and VC in patients who were deceased or had been lung-transplanted. The values for FEV$_1$ and VC at age 5 years (our starting point) which could be inferred from the regression lines were significantly lower in those who were deceased or had been lung-transplanted than in other CF patients, suggesting more severe lung disease early in life.

Chronic colonization with PA has been known for decades to be a predictor of morbidity and mortality. Why CF lungs are so susceptible to *Pseudomonas* infection is still unclear. Speculations about an increased concentration of sodium chloride in CF lungs, which may weaken the innate antibacterial defence system,[22] have been questioned by others.[23] Pier et al.[24] suggested that the CFTR protein itself is a receptor for *Pseudomonas* and is

TABLE 6— Mixed Model Regression Analysis of Rate of Decline in FEV$_1$ vs. Age in Patients With Pancreatic Insufficiency[1]

	Mixed model equation	P-values for differences to reference groups	
		Intercept	Slope
Diabetes (ref. gr)	$Y = 81.7 - 1.232 \times (\text{age} - 5)$		
Nondiabetes	$Y = 86.9 - 0.671 \times (\text{age} - 5)$	$P = 0.12$	$P = 0.02^*$
Cirrhosis (ref. gr)	$Y = 85.0 - 0.880 \times (\text{age} - 5)$		
Noncirrhosis	$Y = 87.3 - 0.803 \times (\text{age} - 5)$	$P = 0.63$	$P = 0.84$

[1]Diabetes and liver cirrhosis were covariates and comparison between subgroups was as detailed in Table 4.
ref. gr, reference group.
*Statistical significance.

directly involved in the clearance of bacteria from the epithelium.

Accordingly, many gene mutations resulting in a functionally defective CFTR protein can be inserted in the cell membrane, but might still be associated with a low risk of *Pseudomonas aeruginosa* infection. In the present study, a significantly lower rate of chronic *Pseudomonas* colonization was found in patients with missense mutations and in patients with pancreatic sufficiency, which supports previous reports indicating that PA infection is influenced by the CFTR genotype.[25]

Results presented here showed a very high frequency of *Pseudomonas* colonization in patients who were deceased or had been lung-transplanted. A significantly faster deterioration in FEV$_1$ was found in PI patients with PA than in those without PA. The large difference between groups that is evident in Figure 5 underscores the detrimental effects of infection with and immune reactions to *Pseudomonas aeruginosa*, and supports results by Parad et al. in 1999.[16] Interestingly, no difference in annual decline of FEV$_1$ was found between PS patients with and without PA colonization. The regression line for patients with PA, however, falls much below the line for those without PA, and the line has a significantly lower intercept value (Fig. 5). This indicates that PS patients with PA may still be at risk for severe lung disease. These PS patients with PA were diagnosed at an older age (mean, 20.1 years) compared with the PS group (mean, 13.7 years) as a whole. The findings raise

Fig. 6. Mixed model regression lines of FEV$_1$ vs. age in years for PI patients with and without diabetes.

several questions. PS patients with PA would probably be in a more favorable state if diagnosed and adequately treated at an earlier age. But the slow decline of lung function, even in these PS/PA patients, may also suggest that PA is more harmless in PS than in PI patients once adequate treatment is provided.

With increasing age, a proportion of CF patients acquire diabetes mellitus. The frequency of diabetes in our CF population was 15.4%, which is comparable with that previously reported.[26] No diabetes was found in the PS patients, which is also consistent with other studies.[26] Progressive clinical deterioration in patients with cystic fibrosis and diabetes mellitus was demonstrated in several reports,[27,28] but not in others.[29] It is debated whether the most severely affected CF patients also are those at greatest risk of developing diabetes mellitus, or whether a prediabetic state contributes to clinical deterioration.[27,30]

The prediabetic state is often associated with being underweight, and a strong association between poor nutrition and poor pulmonary function was found even in non-CF patients.[31,32] In a study by Lanng et al.,[27] weight loss and impairment of pulmonary function could be observed in CF patients 1–4 years before a diagnosis of diabetes mellitus. Insulin therapy could improve lung function and restore body mass index (BMI). Our investigation is, to our knowledge, the first long-term study showing a significantly more rapid decline of FEV$_1$ in diabetic than in nondiabetic patients. No difference was found before age 15 years between patients who later developed diabetes and those who did not. This suggests that it is the diabetes per se, including a prediabetic state, that determines the progression of lung damage. This notion is also supported by Milla et al.,[33] who found a correlation between impaired glucose tolerance and decline of lung function in a group of CF patients followed for 4 years. Consequently, diabetes seems to be a negative predictor for pulmonary function in cystic fibrosis. This finding strengthens the importance of regularly performed oral glucose tolerance tests. Furthermore, it raises the question of whether lung function might benefit from early and aggressive insulin therapy,
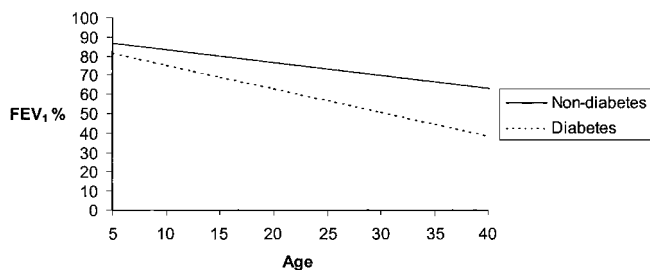
TABLE 7— Relative Risk Ratio of Having FEV₁ Less Than 60% in Different Age Cohorts With Regard to Genotype, Gender, Pancreatic Status, and for Patients With Pancreatic insufficiency Also With Regard to Chronic *Pseudomonas aeruginosia* Infection, Diabetes, and Cirrhosis[1]

Age cohort (years)	Genotype AB vs. C	CL	PI vs. PS	CL	Male vs. female	PA vs. non-PA	CL	Diabetes vs. non-diabetes	CL	Cirrhosis vs. noncirrhosis
≤ 12	NS		1.2	1.1–1.3	NS	3	1.6–6.2	NS		NS
13–18	NS		1.5	1.3–1.6	NS	2.5	1.4–4.6	NS		NS
19–24	3	1.7–5	2.6	1.1–6.5	NS	1.7	1.1–2.8	1.5	1.1–2.2	NS
25–30	NS		NS		NS	3	1.4–7.1	1.5	1.1–2.5	NS

[1]PS, pancreatic sufficiency; NS, not significant; genotype: A, homozygosity for ΔF508; B, severe/severe mutation; C, one or two missense mutations; CL, 95% confidence limits.

as shown by Lanng et al.[27] for patients with diabetes without fasting hyperglycemia. This approach is still not generally recommended.[30]

Whether cirrhosis influences the clinical course of pulmonary disease has not been widely investigated. In a small series of patients with cirrhosis or severe liver fibrosis, a tendency toward better FVC and FEV₁ was found compared with other CF patients.[34] In our analysis, no differences in decline of FEV₁ and VC were found in patients with cirrhosis compared to those without cirrhosis. This suggests that liver cirrhosis per se does not affect pulmonary function.

In conclusion, in the Swedish CF population, the severity of pulmonary disease can be predicted to some extent by CFTR genotype. In patients with pancreatic sufficiency, no significant annual decline of lung function was observed, but those with *Pseudomonas* colonization may still be at risk for serious lung disease. An early diagnosis of patients in this group is desirable. Risk factors that negatively affect lung function included pancreatic insufficiency and diabetes mellitus, in addition to chronic *Pseudomonas* colonization. Patients with these characteristics should receive early and aggressive treatment and close monitoring at CF centers.

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