

# Heritability of Lung Disease Severity in Cystic Fibrosis

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**Rationale:** Obstructive lung disease, the major cause of mortality in cystic fibrosis (CF), is poorly correlated with mutations in the disease-causing gene, indicating that other factors determine severity of lung disease.

**Objectives:** To quantify the contribution of modifier genes to variation in CF lung disease severity.

**Methods:** Pulmonary function data from patients with CF living with their affected twin or sibling were converted into reference values based on both healthy and CF populations. The best measure of FEV<sub>1</sub> within the last year was used for cross-sectional analysis. FEV<sub>1</sub> measures collected over at least 4 years were used for longitudinal analysis. Genetic contribution to disease variation (i.e., heritability) was estimated in two ways: by comparing similarity of lung function in monozygous (MZ) twins (~ 100% gene sharing) with that of dizygous (DZ) twins/siblings (~ 50% gene sharing), and by comparing similarity of lung function measures for related siblings to similarity for all study subjects.

**Measurements and Main Results:** Forty-seven MZ twin pairs, 10 DZ twin pairs, and 231 sibling pairs (of a total of 526 patients) with CF were studied. Correlations for all measures of lung function for MZ twins (0.82–0.91,  $p < 0.0001$ ) were higher than for DZ twins and siblings (0.50–0.64,  $p < 0.001$ ). Heritability estimates from both methods were consistent for each measure of lung function and ranged from 0.54 to 1.0. Heritability estimates generally increased after adjustment for differences in nutritional status (measured as body mass index z-score).

**Conclusions:** Our heritability estimates indicate substantial genetic control of variation in CF lung disease severity, independent of *CFTR* genotype.

**Keywords:** genetics; pulmonary function

Cystic fibrosis (CF) is a lethal autosomal recessive disorder characterized by chronic obstructive pulmonary disease and caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene (1). However, lung function is poorly correlated with *CFTR* mutation (2, 3), indicating that environmental, genetic, and/or stochastic factors are the major determinants of lung disease severity (2, 4). Numerous studies have linked environmental factors, including tobacco smoke exposure (5), bacte-

## AT A GLANCE COMMENTARY

### Scientific Knowledge on the Subject

Severity of cystic fibrosis (CF) lung disease is poorly correlated with *CFTR* mutation. Although numerous candidates have been investigated, the role for modifier genes in CF lung disease severity has not been verified or quantified.

### What This Study Adds to the Field

A significant portion of variability in CF lung disease is due to modifier genes.

rial infection (6, 7), and socioeconomic status (8, 9), with reduced pulmonary function. Conversely, aggressive nutritional intervention has been associated with improved outcomes (10). Although numerous candidate genes have been investigated as CF modifiers (11, 12), a role for genes other than *CFTR* in CF lung disease severity has not been verified or quantified. Family-based studies involving identical (monozygous [MZ]) twins, nonidentical (dizygous [DZ]) twins, and siblings provide an opportunity to assess contribution of genetic and nongenetic factors to disease variation. To this end, we have obtained detailed medical and environmental information from CF twins and CF siblings throughout the United States.

One of the major challenges in any study of the factors underlying disease variation is to accurately define the phenotype. Traditionally, pulmonary function testing (PFT) has been used to determine severity of and monitor progression in CF lung disease (13, 14). The FEV<sub>1</sub> is the PFT measurement that is most predictive of survival in CF (15, 16). Expressing FEV<sub>1</sub> as a percent-predicted value based on a normal reference population illustrates the reduction in pulmonary function of patients with CF relative to healthy individuals (17). However, patients with CF have variable growth abnormalities, including reduced height and delayed puberty (18, 19), that distort the values predicted from healthy populations and complicate comparisons of lung function from one patient with CF to another (20, 21). Disease-specific reference equations for FEV<sub>1</sub> have been developed to compare patients of different age and sex with CF (22, 23). In the current study, the contribution of modifier genes to variation in CF lung disease severity (i.e., heritability) was estimated from cross-sectional and longitudinal measures of lung function derived from both percent-predicted FEV<sub>1</sub> values and from disease-specific measures of FEV<sub>1</sub> in affected twins and siblings. Some of the results of this study have been previously reported in the form of abstracts (24, 25).

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## METHODS

All subjects were recruited on the basis of having an affected sibling. Except for two sets of MZ twins, all patients attended U.S. CF care centers. All patients met CF diagnostic criteria (26). Zygosity for all twin pairs was determined by the AmpFISTR Profiler kit (Applied Biosystems, Foster City, CA). *CFTR* genotype was obtained from medical records, by typing for 31 common CF alleles or by sequencing of the coding and flanking regions (27, 28). Ethnicity was determined by chart review. Written, informed consent or assent was obtained from all subjects.

Pairs of twins or siblings were included in the analysis if both members of the pair had a minimum of four quarterly PFT measurements. A quarter was defined as a 3-month block beginning with the subject's month of birth. PFT data obtained in patients younger than 6 years, after lung transplantation, or when living apart from their affected twin or sibling(s) were excluded. FEV<sub>1</sub> values in liters were converted into percent-predicted values (FEV<sub>1</sub>%pred) (29) and into CF-specific percentiles for FEV<sub>1</sub> (FEV<sub>1</sub>CF%) (23). For cross-sectional analysis, the best FEV<sub>1</sub> measure within the last year of available data was termed MaxFEV<sub>1</sub>CF%. Siblings of different ages were compared using the best FEV<sub>1</sub> for the older sibling from the year when the age of the older sibling matched the current age of the younger sibling. Longitudinal measures were derived from all years of available PFT data for each study subject, using the best FEV<sub>1</sub> measurement per quarter. Rates of change for FEV<sub>1</sub>CF% were calculated by linear regression of FEV<sub>1</sub>CF% on test age in years using FEV<sub>1</sub> data obtained after 1993. The best quarterly FEV<sub>1</sub>CF% for subjects with a minimum of 4 years of PFT data were used to calculate average FEV<sub>1</sub>CF% (AvgFEV<sub>1</sub>CF%). The estimated FEV<sub>1</sub>%pred at 20 years of age (EstFEV<sub>1</sub>%@20yrs) was calculated from a minimum of 5 years of FEV<sub>1</sub> data using mixed modeling and Bayes estimation as described by Schluchter and colleagues (22). The AvgFEV<sub>1</sub>CF% and EstFEV<sub>1</sub>%@20yrs for each individual were used as a single numbers representing lung disease severity over time (longitudinal measures). To assess agreement between the two methods of defining longitudinal lung disease severity, AvgFEV<sub>1</sub>CF% and EstFEV<sub>1</sub>%@20yrs were converted into z-scores based on our patient population. The z-transformed values were compared using Bland-Altman analysis. The average of all body mass index (BMI) z-scores (AvgBMIZ) for each subject was derived from height and weight measurements between 2 and 20 years of age and was used as a longitudinal measure of nutritional status.

Intrapair similarity was determined for MZ twins, DZ twins, and siblings using Pearson pairwise correlation coefficients (*r*). Assignment of twins or siblings as "A" or "B" was permuted 10<sup>6</sup> times. The mean and standard deviation of correlation coefficients obtained from the permutations are reported. Statistical significance of the correlation

coefficients was determined using the corresponding *t* value, calculated using the equation  $t = r/(\sqrt{[(1 - r^2)(n - 2)]})$ . Multiple linear regression was used to determine the contributions of sex, genotype, age at most recent PFT, pancreatic status, and nutritional status to variability in longitudinal measures of lung function. Genotype was defined as 0, 1, or 2 based on the number of  $\Delta F508$  *CFTR* mutations carried by each individual. Pancreatic status was insufficient if the subject had physician-diagnosed pancreatic insufficiency or was taking supplemental pancreatic enzymes. Heritability was estimated by subtracting the correlation coefficient for the combined DZ twin/sibling group from the correlation coefficient for MZ twins and multiplying the difference by two (30). Heritability was also estimated from the siblings alone by dividing additive trait variance among related siblings by total trait variance for all siblings using maximum likelihood estimates as implemented in Sequential Oligogenic Linkage Analysis Routines (SOLAR; <http://www.sfbr.org/solar>) (31). The significance of heritability estimates from SOLAR was determined by likelihood ratio tests, in which the obtained likelihood of the model with the stated additive genetic variance is compared with the likelihood of the model with the additive genetic variance constrained to zero. Statistical calculations and graphing were performed using Intercooled Stata 8 (Stata Corp., College Station, TX). *p* values less than 0.05 were deemed significant. Additional supporting data, including intrapair correlations for  $\Delta F508$  homozygotes and for age-dependent intrapair correlations are included in the online supplement.

## RESULTS

### Demographics

Fifty-seven CF twin pairs and 231 CF sibling pairs (of a total patient population of 526) from 61 fully accredited CF centers and 10 affiliate CF centers throughout the United States and a CF center in Australia were studied (Table 1). One hundred sixty-one subjects were excluded because either the subject or the affected twin or sibling had fewer than four quarterly PFT measurements. The average number of PFT observations per individual was  $23 \pm 14$ , with a range of 4 to 100. The average number of years of PFT data was  $8.8 \pm 5.3$ , with a range of 0.7 to 30.6 years. Four hundred twenty-six patients had at least 4 years of PFT data, and 391 had 5 years of data or more. The average age for the entire group was  $17.4 \pm 7.1$  years, with a range of 6.8 to 46.7 years. The MZ twins were slightly older than the DZ twins and siblings, although age ranges for the different groups overlapped considerably (Table 1). Males represented 54.2% of the

TABLE 1. CHARACTERISTICS OF STUDY SUBJECTS

	MZ Twins	DZ Twins	Siblings	Combined DZ Twins/Siblings*
Individuals	94 (47 pairs)	20 (10 pairs)	412 (231 pairs) <sup>†</sup>	149 (79 pairs) <sup>‡</sup>
Average age $\pm$ SD at most recent PFT (range)	20.2 $\pm$ 8.2 yrs (7.8–46.7 yr)	17.6 $\pm$ 7.1 yrs (10.8–34.5 yrs)	16.8 $\pm$ 6.7 yrs (6.8–46.0 yrs)	16.9 $\pm$ 6.3 (7.8–39.0 yrs)
Sex				
Female	44	7	190	59
Male	50	13	222	90
Pancreatic status <sup>§</sup>				
Insufficient	93	19	368	139
Sufficient	1	1	43	9
Unknown	0	0	1	1
<i>CFTR</i> genotype				
$\Delta F508/\Delta F508$	56 (59.6%)	10 (50%)	203 (49.3%)	75 (50.3%)
Non- $\Delta F508$ homozygotes	38 (40.4%)	10 (50%)	209 (50.7%)	74 (49.7%)

Definition of abbreviations: DZ = dizygous; MZ = monozygous; PFT = pulmonary function testing.

\* Same-sex DZ twins and same-sex siblings with fewer than 3 years' difference in age.

<sup>†</sup> Fourteen families with three affected children (counted as three pairs), one family with four affected children (counted as six pairs).

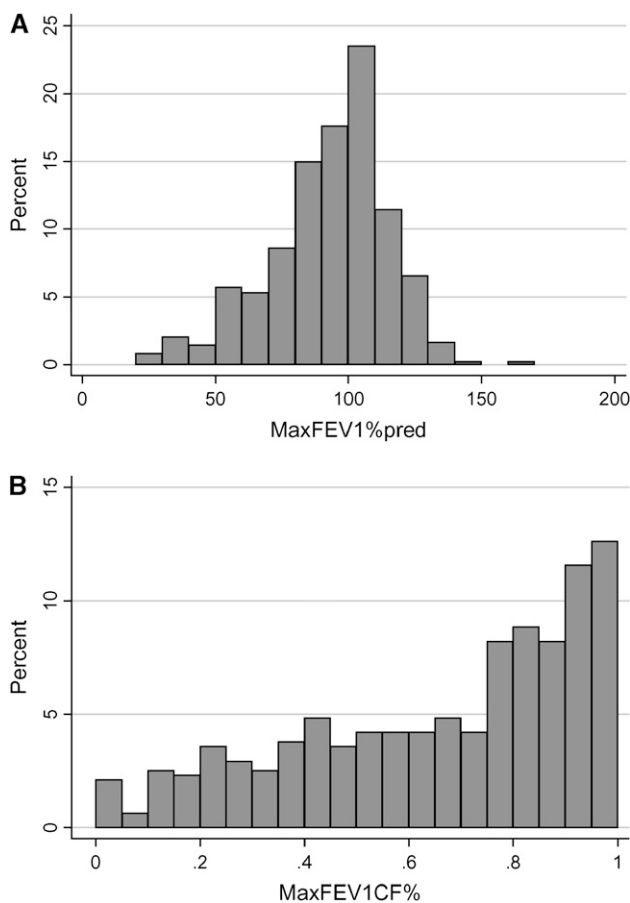
<sup>‡</sup> Three families with three affected children (counted as three pairs).

<sup>§</sup> Physician-diagnosed pancreatic insufficiency or individual taking supplemental pancreatic enzymes.

total study population. The majority (91.3%) of subjects had pancreatic insufficiency. Individuals homozygous for the  $\Delta F508$  *CFTR* mutation represented 51.4% of the entire study population. The distribution of  $\Delta F508$  homozygotes within each class is within expected variance given the sample size. Most subjects were white (89.9%), whereas a small minority were Hispanic/Latino (2.7%), African American (1.1%), Asian (0.6%), Middle Eastern (0.4%), or of mixed racial descent (3.0%). Ethnicity was unknown for 2.3% of study subjects. The demographic features of the study subjects mirror those of the population of patients with CF in the United States in 2004, except for a younger mean age (32).

### Measures of the Severity of CF Lung Disease

The distribution of the cross-sectional measures of lung function in the study population is shown in Figure 1. The mean  $\text{MaxFEV}_1\% \text{pred}$  for the study patients was  $87.8\% (\pm 25.7; \text{range}, 16\text{--}165)$ . The mean  $\text{MaxFEV}_1\text{CF}\%$  for the study patients was  $0.66 (\pm 0.29; \text{range}, 0\text{--}1)$ . Because the CF-specific percentiles



**Figure 1.** Distribution of the cross-sectional measures of lung function in the cystic fibrosis (CF) twins and siblings. (A) The x axis represents  $\text{FEV}_1$  expressed as percent-predicted based on the equations of Knudson ( $\text{MaxFEV}_1\% \text{pred}$ ), in increments of 10%. The y axis shows the percentage of study patients with a  $\text{MaxFEV}_1\% \text{pred}$  that falls within each 10% increment. (B)  $\text{FEV}_1$  expressed as CF-specific percentile based on the equations of Kulich ( $\text{FEV}_1\text{CF}\%$ ), in increments of 0.05. The y axis shows the percentage of study patients with an  $\text{FEV}_1\text{CF}\%$  that falls within each 0.05 increment.  $\text{FEV}_1\text{CF}\%$  is expressed as a fraction, with 0 corresponding to the most severe lung disease and 1 corresponding to the mildest lung disease (i.e., the equivalent of the 100th percentile).

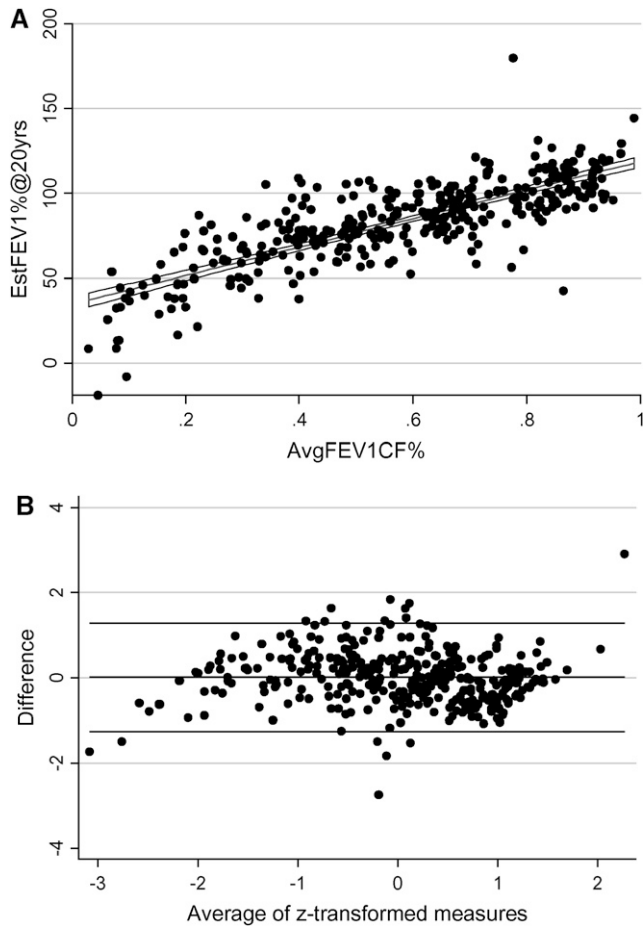
for  $\text{FEV}_1$  represent the entire CF population, an increment of 0.05 would be expected to represent 5% of patients if the disease severity of the study population exactly mirrored that of the CF population as a whole. The study subjects encompass the entire spectrum of severity, although a substantial fraction have moderate to mild lung disease, compared with the entire CF population.

To determine if  $\text{FEV}_1\text{CF}\%$  changes over time, we plotted the best quarterly  $\text{FEV}_1\text{CF}\%$  for all study subjects versus age at time of PFT measurement. The mean linear rate of change for the entire group was  $0.00 \pm 0.03$ . The rate of change in  $\text{FEV}_1\text{CF}\%$  was between  $-0.03$  and  $0.03$  for 68% of study subjects, and 98% had a rate of change of less than 0.10 per year. To evaluate the ability of  $\text{AvgFEV}_1\text{CF}\%$  to predict the actual  $\text{FEV}_1\text{CF}\%$  at age 20, we compared  $\text{AvgFEV}_1\text{CF}\%$  to the known  $\text{MaxFEV}_1\text{CF}\%$  at age 20 for the 120 subjects for whom these data were available. The average absolute difference in actual and  $\text{AvgFEV}_1\text{CF}\%$  was  $0.096 \pm 0.087$  (range, 0.002–0.578). The majority (64.7%) of the subjects had a  $\text{MaxFEV}_1\text{CF}\%$  at age 20 that differed by less than 0.10 from the  $\text{AvgFEV}_1\text{CF}\%$ , and 89.9% differed by less than 0.20. Because  $\text{FEV}_1\text{CF}\%$  remains relatively stable for a number of years for many patients with CF and is predictive of lung function at age 20, we chose to use  $\text{AvgFEV}_1\text{CF}\%$  as a longitudinal measurement of the severity of lung disease. A Bayesian model that predicts  $\text{FEV}_1$  at 20 years of age ( $\text{EstFEV}_1\% @ 20\text{yrs}$ ) was used as a second longitudinal measure of lung function (22). To evaluate the validity of this model for our population, we compared the known value of  $\text{FEV}_1\% \text{pred}$  for the 87 subjects who had a PFT measurement at age 20 with the value predicted by the model. The mean absolute difference between the  $\text{EstFEV}_1\% @ 20\text{yrs}$  and the  $\text{MaxFEV}_1\% \text{pred}$  at age 20 was  $10.2 \pm 15.2$ , with a range of 0.1 to 97.5. The majority (69%) of subjects had predicted values that differed from actual values by 10% or less, and 87.4% differed by less than 20%. These two longitudinal models of CF lung disease were highly correlated ( $r = 0.80, p < 0.0001$ ) for the 341 individuals for whom both measures were available (Figure 2A). When considering z-transformed longitudinal measures, the mean difference between the two measurements is small. However, there is wide variation in difference between the two measures for any given mean, suggesting that the two longitudinal measures represent slightly different aspects of lung disease severity. There is no systematic bias between the two measures (Figure 2B).

### Covariate Analysis

Previous studies evaluating genetic contribution to variability in longitudinal measures of pulmonary function have found pulmonary function to be closely related to nutritional status (10, 33, 34). We used the  $\text{AvgBMIZ}$  as an estimate of nutritional status. Regression analysis was performed to evaluate the contributions of  $\text{AvgBMIZ}$ , pancreatic status, genotype, and age at most recent PFT measurement (maximum test age) to variability in longitudinal measures of severity of CF lung disease. When considered independently,  $\text{AvgBMIZ}$  and pancreatic status were significant covariates of  $\text{EstFEV}_1\% @ 20\text{yrs}$  ( $p < 0.001$ ), whereas  $\text{AvgBMIZ}$  was the only significant covariate for  $\text{AvgFEV}_1\text{CF}\%$  ( $p < 0.001$ ). When all covariates were included in a single model using multiple linear regression,  $\text{AvgBMIZ}$  remained a highly significant covariate for both measures, whereas pancreatic status was also a significant covariate for  $\text{EstFEV}_1\% @ 20\text{yrs}$  (Table 2). The best-fit model for  $\text{EstFEV}_1\% @ 20\text{yrs}$  was  $88.4 + 13.8$  (if pancreatic sufficient)  $+ (15.4 \times \text{AvgBMIZ})$ . For  $\text{AvgFEV}_1\text{CF}\%$ , the best-fit model was  $0.626 + (0.124 \times \text{AvgBMIZ})$ .  $\text{AvgBMIZ}$  accounts for 20% of the total variation in  $\text{AvgFEV}_1\text{CF}\%$  and 28% of the total variation in  $\text{EstFEV}_1\% @ 20\text{yrs}$ .





**Figure 2.** Agreement between longitudinal measures of lung function. (A) Each point on the graph represents an individual subject with a minimum of 5 years of pulmonary function testing data while living at home with an affected sibling. The x axis represents the average FEV<sub>1</sub>CF% (AvgFEV<sub>1</sub>CF%) and the y axis represents the estimated FEV<sub>1</sub>% at 20 years (EstFEV<sub>1</sub>%@20yrs) for each individual. The best-fit line with 95% confidence intervals are shown. (B) Each point on this Bland-Altman plot represents a single subject. The x axis represents the mean of the two longitudinal measures and the y axis represents the difference between the two longitudinal measures for each subject. The horizontal lines on the graph represent the mean difference between the two measures for all subjects (center line) and +2 and -2 standard deviations from that mean (upper and lower horizontal lines, respectively).

### Estimation of Genetic Effect

The intrapair correlations of the longitudinal measure of CF-specific lung function AvgFEV<sub>1</sub>CF% for MZ twins, DZ twins, siblings, and the combined group are shown in Figure 3. Similar trends were observed for the cross-sectional measure MaxFEV<sub>1</sub>CF% and the other longitudinal measure of EstFEV<sub>1</sub>%@20yrs (Table 3). The combined group of same-sex DZ twins and same-sex siblings within 3 years of age had similar or higher correlation than the entire group of siblings for each measurement (Table 3). To assess the effect of age on intrapair similarity, we calculated correlations for MZ twin and combined DZ twin/sibling pairs who were both younger than 15 years and for pairs who were both older than 15 years. Intrapair similarity for the younger and the older pairs did not differ significantly (see Table E1 in the online supplement). The high correlation among MZ twin pairs (~100% gene sharing) compared with DZ twin pairs

**TABLE 2. THE MAGNITUDE AND SIGNIFICANCE OF FACTORS INFLUENCING LONGITUDINAL MEASURES OF LUNG FUNCTION**

	Coefficient*	95% CI†	p Value‡
EstFEV <sub>1</sub> %@20yrs			
AvgBMIZ	15.6 ± 1.6	12.5 to 18.7	< 0.001
ΔF508	-3.4 ± 2.2	-7.7 to 1.0	0.132
Pancreatic status	11.2 ± 5.8	-0.2 to 22.7	0.053
Max. test age	0.2 ± 0.3	-0.4 to 0.7	0.550
Constant	90.7 ± 6.0	78.8 to 102.6	< 0.001
Best-fit model: EstFEV <sub>1</sub> %@20yrs = 88.4 + 13.8 (if pancreatic sufficient) + [15.46 × AvgBMIZ]			
AvgFEV <sub>1</sub> CF%			
AvgBMIZ	0.126 ± 0.013	0.100 to 0.152	< 0.001
ΔF508	0.001 ± 0.019	-0.036 to 0.038	0.972
Pancreatic status	0.034 ± 0.051	-0.067 to 0.134	0.508
Max. test age	0.004 ± 0.002	-0.000 to 0.008	0.074
Constant	0.555 ± 0.048	0.460 to 0.649	< 0.001
Best-fit model: AvgFEV <sub>1</sub> CF% = 0.626 + [0.124 × AvgBMIZ]			

*Definition of abbreviations:* AvgBMIZ = average of all body mass index z-scores; AvgFEV<sub>1</sub>CF% = average cystic fibrosis-specific percentile for FEV<sub>1</sub>; CI = confidence interval; EstFEV<sub>1</sub>%@20yrs = estimated FEV<sub>1</sub>%pred at 20 years.

\* Coefficient is the magnitude of the effect of each factor derived from multivariate analysis. Standard deviation for each coefficient is shown.

† The 95% confidence interval for each coefficient.

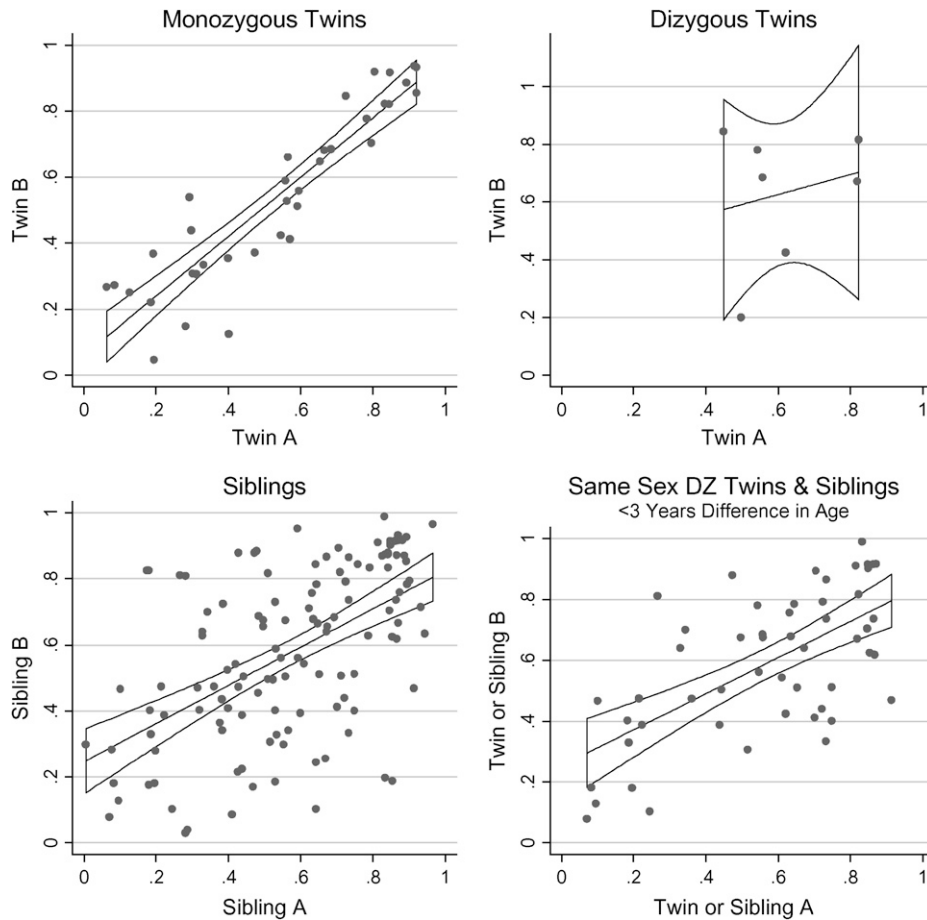
‡ p value refers to significance of the coefficient.

and sibling pairs (~50% gene sharing) indicates strong genetic contribution to variation in each measure of lung function (Table 4). Estimates of heritability for longitudinal measures increased after adjusting for their significant covariates. Using the same techniques described above, correlations were calculated for twins and siblings homozygous for the common *CFTR* mutation ΔF508 (see Table E2). Twins and siblings homozygous for ΔF508 demonstrate strong genetic control of variation in lung disease (Table 4). Heritability estimates from siblings using variance components methods also demonstrate substantial genetic contribution to variation in lung function (Table 4). Estimates obtained from the sibling analysis were generally equal to or higher than those obtained by comparing MZ twins to DZ twins and siblings.

### DISCUSSION

Identifying the underlying causes of variation in lung disease severity is a major goal of CF research. The discovery of the *CFTR* gene and characterization of its mutant alleles revealed that pancreatic status and, to some degree, sweat gland dysfunction are sensitive to variability in *CFTR* function (2, 35). However, *CFTR* genotype correlates poorly with pulmonary phenotype (36). Realization of the latter combined with the challenge posed by *CFTR* replacement therapy has intensified study of the mechanisms responsible for progression of obstructive airway disease, the primary cause of morbidity and mortality in patients with CF. Affected twins and siblings demonstrate that genetic control of both cross-sectional and longitudinal measures of lung function is substantial. The results of this study validate searches for CF modifier genes and, more importantly, provide a basis to quantify the contribution of identified modifiers to the heritable fraction of variation in pulmonary function. This discovery should lead to new insights into the pathophysiology of CF lung disease, and ultimately to development of new CF therapies.

The related patients with CF in this study are representative of the wide spectrum of disease severity observed in the entire CF population. However, the CF twins and siblings have better lung function than the measures reported to the CF Foundation



**Figure 3.** Correlation of the longitudinal measure of CF-specific lung function in twins and siblings. For each plot, the x axis represents the AvgFEV<sub>1</sub>CF% for twin or sibling A and the y axis represents the AvgFEV<sub>1</sub>CF% for twin or sibling B. The points on each graph represent one twin pair or one sibling pair. The best-fit lines with 95% confidence intervals are shown. DZ = dizygous.

patient registry by CF care centers in the United States (32). We used the best quarterly FEV<sub>1</sub> measures to minimize variability in FEV<sub>1</sub> measures due to intercurrent illnesses, insufficient patient effort, or inherent test variability. Although CF centers typically

report the best FEV<sub>1</sub>, we cannot verify that the CF Foundation patient registry represents only optimum PFT measures. The bias toward milder lung disease could also be a consequence of recruiting only patients with CF who have at least one surviving

**TABLE 3. INTRAPAIR CORRELATIONS FOR CROSS-SECTIONAL AND LONGITUDINAL MEASURES OF CF LUNG DISEASE SEVERITY IN TWINS AND SIBLINGS LIVING TOGETHER**

	MaxFEV <sub>1</sub> CF% (n)	EstFEV <sub>1</sub> %@20yrs* (n)	Adjusted <sup>†</sup> EstFEV <sub>1</sub> %@20yrs (n)	AvgFEV <sub>1</sub> CF% <sup>‡</sup> (n)	Adjusted <sup>§</sup> AvgFEV <sub>1</sub> CF% (n)
MZ twins	0.88 ± 0.08 <sup>  </sup> (38)	0.81 ± 0.01 <sup>  </sup> (34)	0.80 ± 0.01 <sup>  </sup> (34)	0.91 ± 0.01 <sup>  </sup> (36)	0.93 ± 0.00 <sup>  </sup> (32)
DZ twins	0.58 ± 0.08 (8)	0.16 ± 0.11 (7)	0.49 ± 0.09 (7)	0.30 ± 0.12 (7)	0.65 ± 0.07 (7)
Siblings	0.36 ± 0.00 <sup>  </sup> (184)	0.41 ± 0.01 <sup>‡</sup> (90)	0.43 ± 0.01 <sup>  </sup> (87)	0.55 ± 0.00 <sup>  </sup> (124)	0.49 ± 0.00 <sup>  </sup> (117)
Same-sex Siblings, < 3 yr difference in age	0.53 ± 0.01 <sup>  </sup> (61)	0.51 ± 0.03 <sup>**</sup> (37)	0.36 ± 0.03 (37)	0.65 ± 0.01 <sup>  </sup> (47)	0.54 ± 0.02 <sup>**</sup> (45)
Same-sex DZ twins and siblings, < 3 yr difference in age	0.54 ± 0.03 <sup>  </sup> (67)	0.50 ± 0.02 <sup>‡</sup> (42)	0.40 ± 0.03 <sup>††</sup> (42)	0.64 ± 0.07 <sup>  </sup> (52)	0.58 ± 0.06 <sup>  </sup> (50)

*Definition of abbreviations:* AvgBMIZ = average of all body mass index z-scores; AvgFEV<sub>1</sub>CF% = average cystic fibrosis-specific percentile for FEV<sub>1</sub>; CI = confidence interval; DZ = dizygous; EstFEV<sub>1</sub>%@20yrs = estimated FEV<sub>1</sub>%pred at 20 years; MaxFEV<sub>1</sub>CF% = best cystic fibrosis-specific percentile for FEV<sub>1</sub> within the last year of available data; MZ = monozygous.

\* Using minimum of 5 years of PFT data.

<sup>†</sup> EstFEV<sub>1</sub>%@20yrs adjusted for AvgBMIZ and for pancreatic status.

<sup>‡</sup> Using minimum of 4 years of PFT data.

<sup>§</sup> AvgFEV<sub>1</sub>CF% adjusted for AvgBMIZ.

<sup>||</sup> p value < 0.0001.

<sup>‡</sup> p value < 0.001.

<sup>\*\*</sup> p value < 0.005.

<sup>††</sup> p value < 0.01.

**TABLE 4. HERITABILITY ESTIMATES FOR CROSS-SECTIONAL AND LONGITUDINAL MEASURES OF LUNG FUNCTION**

	Twins and Siblings*		All Siblings <sup>†</sup> (SE)	
	All Subjects	ΔF508	All Subjects	ΔF508
		Homozygotes		Homozygotes
MaxFEV <sub>1</sub> CF%	0.68	0.86	0.68 (0.14) <sup>‡</sup>	0.54 (0.22) <sup>§</sup>
AvgFEV <sub>1</sub> CF%	0.54	0.80	1.00 (no SE) <sup>‡</sup>	0.96 (0.19) <sup>‡</sup>
AdjAvgFEV <sub>1</sub> CF%	0.70	0.78	0.96 (0.14) <sup>‡</sup>	0.89 (0.17) <sup>‡</sup>
EstFEV <sub>1</sub> %@20yrs	0.62	0.56	0.73 (0.19) <sup>§</sup>	0.65 (0.28)
AdjEstFEV <sub>1</sub> %@20yrs	0.82	0.76	0.83 (0.20) <sup>§</sup>	1.00 (no SE) <sup>‡</sup>

*Definition of abbreviations:* AdjAvgFEV<sub>1</sub>CF% = adjusted average cystic fibrosis-specific percentile for FEV<sub>1</sub>; AdjEstFEV<sub>1</sub>%@20yrs = adjusted estimated FEV<sub>1</sub>%pred at 20 years; AvgFEV<sub>1</sub>CF% = average cystic fibrosis-specific percentile for FEV<sub>1</sub>; EstFEV<sub>1</sub>%@20yrs = estimated FEV<sub>1</sub>%pred at 20 years; MaxFEV<sub>1</sub>CF% = best cystic fibrosis-specific percentile for FEV<sub>1</sub> within the last year of available data.

\* Heritability estimated by multiplying by 2 the correlation in MZ twins minus the correlation in combined same-sex DZ twin and same-sex siblings with < 3 years' difference in age (30)

<sup>†</sup> Heritability estimated by dividing additive trait variance among related siblings by total trait variance for the entire group of siblings using maximum likelihood estimates as implemented in Sequential Oligogenic Linkage Analysis Routines (SOLAR) (31). Standard errors for each estimate are shown in parentheses.

<sup>‡</sup> p value < 0.0001.

<sup>§</sup> p value < 0.001.

sibling, thereby excluding siblings of deceased patients who potentially have more severe disease and the severely affected offspring whose parents elected to forego additional childbearing. The genetic contribution to early and severe lung disease is unknown. For many conditions, early-onset, severe disease usually has a higher likelihood of significant genetic effect (37). Absence of some sibling pairs with severe disease might have reduced estimates of genetic effect. On the other hand, the estimates of genetic effect presented here could be inflated. First, this study had an insufficient number of DZ twins from which to derive meaningful estimates of intrapair similarity. For this reason, we combined the DZ twins with siblings to achieve robust correlation coefficients. However, unlike DZ twins, siblings do not share an *in utero* environment nor does their home environment exactly match that of their sibling during critical periods of lung development. Using siblings as a proxy for DZ twins may have substantially lowered correlations from "actual" levels among those sharing 50% of their genes, thereby inflating heritability estimates. Second, it is plausible that MZ twins have higher levels of shared environment than DZ twins or siblings by virtue of their "identical" status (38). Although experimental evidence from behavioral studies counters this argument (39, 40), we did not test for differences in shared environment among twin pairs. Finally, error in estimating heritability from twins and siblings can arise from differences in the distribution of phenotypes among the groups of related patients (41). To minimize this source of inaccuracy, heritability was estimated only for lung function measures that did not differ significantly ( $p > 0.2$ ) in means and variances between the MZ and combined DZ twin/sibling groups.

Correlation of all measures of lung function for MZ twins were high but were not 100%, suggesting a role for environmental and/or stochastic factors in CF lung disease variation. To minimize difference in environmental factors among twins and siblings, we analyzed lung function data collected while study subjects were living at home with their affected twin or sibling. Shared home environment is likely to control for significant environmental covariates, such as socioeconomic status (8, 9), ambient air pollution (42), and tobacco smoke (5). However, the increase in correlation coefficients in siblings selected for

same sex and similarity in age suggests that there are additional sources of variation, even in a shared home environment. Future goals for this project will be to investigate the contribution of unique environmental factors, such as infection history, compliance with treatment regimens, and tobacco use to variation in CF lung disease.

The two predictive models for lung disease progression used in this study were derived from different CF populations, yet had similar predictive power and were highly correlated. Bland-Altman analysis of agreement between the two measures, however, indicates wide variation between the two methods of defining lung disease severity. This fact may be explained partially by the populations from which the two models were derived. EstFEV<sub>1</sub>%@20yrs was based on lung function data from 188 ΔF508 homozygotes born after 1965 and monitored at a single center (22). In contrast, FEV<sub>1</sub>CF% values were based on more than 25,000 patients with a variety of *CFTR* genotypes monitored from 1994 to 2001 at centers throughout the United States (23). Although EstFEV<sub>1</sub>%@20yrs was derived from ΔF508 patients only, *CFTR* genotype was not a significant covariate for this measure in our subjects. This finding is likely explained by the significance of pancreatic status to the EstFEV<sub>1</sub>%@20yrs model and the observation that *CFTR* genotype is highly correlated with pancreatic function (2). The similarity of these longitudinal prediction models may be explained by relative homogeneity in patterns of disease progression in CF. Indeed, different samples of the CF population have reported similar annual rates of decline in FEV<sub>1</sub>%pred, with mean values ranging from -1.5 to -3.6 (7, 14, 43-46). The results presented here indicate that AvgFEV<sub>1</sub>CF% or EstFEV<sub>1</sub>%@20yrs corrected for pancreatic status can be used to test genes that are candidate modifiers of CF lung disease.

Nutritional status has been shown to be associated with severity of lung disease in CF, but the exact nature of the relationship between nutritional status and lung function is unknown (10, 18, 33, 34). Evidence of genetic influence on both traits was reported by the European CF Twin and Sibling Study when the investigators noted that concordance for a composite cross-sectional measure of lung function and nutritional status was higher in 29 MZ twin pairs than in 12 DZ twin pairs ( $p < 0.04$ ) (47). However, concordance rates did not differ when lung function and nutritional status were considered independently (47). Furthermore, genetic effect on longitudinal measures was not evaluated (47). Recently, Drumm and colleagues associated alleles of TGF-β1 with lung disease severity in patients with CF (12). The dichotomization strategy used to group patients by lung function measures also segregated patients by nutritional status (12). The Drumm and colleagues' study did not discern whether TGF-β1 alleles were associated with severity in lung disease, malnutrition, or both. Regression analysis was used here to quantify the interrelatedness of lung function and nutritional status. Longitudinal lung function measures adjusted for variation in nutritional status were less similar in pairs of DZ twins and siblings than unadjusted measures. On the other hand, pairs of MZ twins were very similar for unadjusted and adjusted measures. Thus, differences in nutritional status caused a fraction of the pairs of DZ twins and siblings to appear to have similar lung function, whereas nearly all of the MZ twins were similar in both respects. The disparity between the MZ and DZ/sibling groups indicates the presence of factors, possibly genetic, that modulate nutritional status independent of the genetic modifiers of lung function.

Studies of healthy individuals suggest that genes play a significant role in determining FEV<sub>1</sub>, even among individuals in different environments. Estimates of heritability obtained for cross-sectional FEV<sub>1</sub> in various healthy adult populations



(0.5–0.77) are comparable to our estimates from individuals with CF (0.68) (48–51). Whether the same or different genes contribute to cross-sectional measures of lung function in healthy individuals and those with a chronic and progressive obstructive disorder such as CF remains to be determined. Likewise, genes that influence lung function over time may differ from those that determine cross-sectional measures. Only one study evaluating genetic effect on longitudinal measures of lung function in healthy individuals has been published (52). The aforementioned study used FEV<sub>1</sub> measured at two time points to derive linear rates of change and demonstrated only small genetic effect. As shown here, longitudinal measures derived from modeling disclosed strong genetic control of the progression of CF lung disease. If pulmonary response to chronic injury follows predictable genetically determined paths, then similar processes may underlie loss of lung function in the more common complex lung diseases.

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