

Heritability of Lung Disease Severity in Cystic Fibrosis

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Significance: Severity of CF lung disease is poorly correlated with CFTR mutation. Though numerous candidates have been investigated, the role for modifier genes in CF lung disease severity has not been verified or quantified. This study demonstrates that a significant portion of variability in CF lung disease is due to modifier genes. This result also provides a basis to quantify the contribution of modifiers genes to variation in pulmonary function.

Running Heading: Heritability of CF Lung Disease Severity

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Additional supporting data, including intra-pair correlations for $\Delta F508$ homozygotes and for age-dependent intra-pair correlations are included in the online supplement.

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ABSTRACT

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 CF patients living with their affected twin or sibling were converted into reference values based on both healthy and CF populations. The best measure of forced expiratory volume in 1 second (FEV1) within the last year was used for cross-sectional analysis. FEV1 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 MZ twins (~100% gene sharing) to that of 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: 47 MZ twin pairs, 10 DZ twin pairs, and 231 sibling pairs (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.

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INTRODUCTION

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*) gene¹. 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 exposure⁵, bacterial infection^{6;7} and socioeconomic status^{8;9} with reduced pulmonary function. Conversely, aggressive nutritional intervention has been associated with improved outcomes¹⁰. 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) twins, non-identical (dizygous) twins and siblings provide an opportunity to assess contribution of genetic and non-genetic 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 forced expiratory volume in one second (FEV1) is the PFT measurement that is most predictive of survival in CF^{15;16}. Expressing FEV1 as a percent-predicted value based on a normal reference population illustrates the reduction in pulmonary function of CF patients relative to healthy individuals¹⁷. However, CF patients 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 CF patient to another^{20;21}. Disease-specific reference equations for FEV1 have been developed to compare CF patients of different age and gender^{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 FEV1 values and from disease-specific measures of FEV1 in affected twins and siblings. Some of the results of this study have been previously reported in the form of abstracts^{24;25}.

MATERIALS AND METHODS:

All subjects were recruited on the basis of having an affected sibling. Except for two sets of monozygous twins, all patients attended US CF care centers. All patients met CF diagnostic criteria²⁶. Zygosity for all twin pairs was determined by the AmpF \mathcal{L} STR 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 regions 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 4 quarterly PFT measurements. A quarter was defined as a 3 month block beginning with the subject's month of birth. PFT data obtained before the age of 6 years, after lung transplantation, or when living apart from their affected twin or sibling(s) were excluded. FEV1 values in liters were converted into percent predicted values (FEV1%pred)²⁹ and into CF-specific percentiles for FEV1 (FEV1CF%)²³. For cross-sectional analysis, the best FEV1 measure within

the last year of available data was termed MaxFEV1CF%. Siblings of different ages were compared using the best FEV1 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 FEV1 measurement per quarter. Rates of change for FEV1CF% were calculated by linear regression of FEV1CF% on test age in years using FEV1 data obtained after 1993. The best quarterly FEV1CF%_s for subjects with a minimum of 4 years of PFT data were used to calculate average FEV1CF% (AvgFEV1CF%). The estimated FEV1%_{pred} at 20 years of age (EstFEV1%_{@20yrs}) was calculated from a minimum of 5 years of FEV1 data using mixed modeling and bayes estimation as described by Schluchter, et al ²². The AvgFEV1CF% and EstFEV1%_{@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, AvgFEV1CF% and EstFEV1%_{@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 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.

Intra-pair similarity was determined for MZ twins, DZ twins, and siblings using Pearson pair-wise correlation coefficients (r). Assignment of twins or siblings as “A” or “B” was permuted 10⁶ 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 $t = r / (\text{sqrt}[(1-r^2)(N-2)])$. Multiple

linear regression was used to determine the contributions of gender, 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 two³⁰. 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. 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 to the likelihood of the model with the additive genetic variance constrained to zero. Statistical calculations and graphing were performed using Intercooled Stata 8[®]. P-values less than 0.05 were deemed significant.

RESULTS

Demographics

Fifty-seven CF twin pairs and 231 CF sibling pairs (526 patients) from 61 fully accredited CF centers and 10 affiliate CF centers throughout the U.S and a CF center in Australia were studied (Table 1). 161 subjects were excluded because either the subject or the affected twin or sibling had less than 4 quarterly PFT measurements. The average number of PFT observations per individual was 23 ± 14 , with a range of 4 to 100. The average number of years of PFT data was

8.8 ± 5.3, with a range of 0.7 to 30.6 years. 426 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 ± 7.1 years, with a range of 6.8 to 46.7 years. The monozygous (MZ) twins were slightly older than the dizygous (DZ) twins and siblings, though age ranges for the different groups overlapped considerably (Table 1). Males represented 54.2% of the total. The majority (91.3%) of subjects were pancreatic insufficient. 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 sample size. Most subjects were Caucasian (89.9%), while 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 CF patients in the United States in 2004 except for a younger mean age³².

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 MaxFEV1%pred for the study patients was 87.8% (± 25.7 , range 16-165). The mean MaxFEV1CF% for the study patients was 0.66 (± 0.29 , range 0-1). Because the CF-specific percentiles for FEV1 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 to the entire CF population.

To determine if FEV1CF% changes over time, we plotted the best quarterly FEV1CF% for all study subjects versus age at time of PFT measurement. The mean linear rate of change for the entire group was 0.00 ± 0.03 . The rate of change in FEV1CF% 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 AvgFEV1CF% to predict the actual FEV1CF% at age 20, we compared AvgFEV1CF% to the known MaxFEV1CF% at age 20 for the 120 subjects for whom this data was available. The average absolute difference in actual and AvgFEV1CF% was 0.096 ± 0.087 (range 0.002 to 0.578). The majority (64.7%) of the subjects had a MaxFEV1CF% at age 20 that differed by less than 0.10 from the AvgFEV1CF%, and 89.9% differed by less than 0.20. Since FEV1CF% remains relatively stable for a number of years for many CF patients and is predictive of lung function at age 20, we chose to use AvgFEV1CF% as a longitudinal measurement of the severity of lung disease. A Bayesian model that predicts FEV1 at 20 years of age (EstFEV1%@20yrs) was used as a second longitudinal measure of lung function²². To evaluate the validity of this model for our population, we compared the known value of FEV1%pred for the 87 subjects who had a PFT measurement at age 20 to the value predicted by the model. The mean absolute difference between the EstFEV1%@20yrs and the MaxFEV1%pred at age 20 was 10.2 ± 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 average BMI Z-score (AvgBMIZ) as an estimate of nutritional status.

Regression analysis was performed to evaluate the contributions of 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, AvgBMIZ and pancreatic status were significant covariates of EstFEV1%@20yrs ($p < 0.001$) while AvgBMIZ was the only significant covariate for AvgFEV1CF% ($p < 0.001$). When all covariates were included in a single model using multiple linear regression, AvgBMIZ remained a highly significant covariate for both measures while pancreatic status was also a significant covariate for EstFEV1%@20yrs (Table 2). The best fit model for EstFEV1%@20yrs was $88.4 + (13.8 \text{ if pancreatic sufficient}) + (15.4 \times \text{AvgBMIZ})$. For AvgFEV1CF%, the best fit model was $0.626 + (0.124 \times \text{AvgBMIZ})$. AvgBMIZ accounts for 20% of the total variation in AvgFEVCF% and 28% of the total variation in EstFEV1%@20yrs.

Estimation of genetic effect

The intra-pair correlations of the longitudinal measure of CF-specific lung function AvgFEV1CF% for MZ twins, DZ twins, siblings and the combined group are shown in Figure 3. Similar trends were observed for the cross-sectional measure MaxFEV1CF% and the other

longitudinal measure EstFEV1%@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 intra-pair similarity, we calculated correlations for MZ twin and combined DZ twin/sibling pairs who were both less than 15 years of age and for pairs who were both greater than 15 years of age. Intra-pair similarity for the younger and the older pairs did not differ significantly (see Table E1 in the online data supplement). The high correlation among MZ twin pairs (~100% gene sharing) compared to DZ twin pairs 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 $\Delta F508$ (see Table E2 in the online data supplement). Twins and siblings homozygous for $\Delta F508$ 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 phenotype³⁶.

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 CF patients. 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 CF patients 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 patient registry by CF care centers in the US³². We used the best quarterly FEV1 measures to minimize variability in FEV1 measures due to intercurrent illnesses, insufficient patient effort, or inherent test variability. Although CF centers typically report the best FEV1, 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 CF patients with at least one surviving 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 effect³⁷. 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 intra-pair 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” status³⁸. 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 patients⁴¹. 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 pollution⁴² and tobacco smoke⁵. 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. EstFEV1%@20yrs was based upon lung function data from 188 Δ F508 homozygotes born after 1965 and followed at a single center²². In contrast, FEV1CF%s were based upon more than 25,000 patients with a variety of CFTR genotypes followed from 1994 to 2001 at centers throughout the US²³. Although EstFEV1%@20yrs was derived from Δ F508 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 EstFEV1%@20yrs model and the observation that CFTR genotype is highly correlated with pancreatic function². 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 FEV1%pred, with mean values ranging from -1.5 to -3.6^{7;14;43-46}. The results presented here indicate that AvgFEV1CF% or EstFEV1%@20yrs corrected for pancreatic status can be employed 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 cystic fibrosis, but the exact nature of the relationship between nutritional status and lung function is unknown^{10;18;33;34}. Evidence of genetic influence upon both traits was reported by the European CF Twin and Sibling Study when they 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 independently⁴⁷. Furthermore, genetic effect upon longitudinal measures was not evaluated⁴⁷. Recently, Drumm and colleagues associated alleles of TGF β 1 with lung disease severity in CF patients¹². The dichotomization strategy used to group patients by lung function measures also segregated patients by nutritional status¹². The Drumm study did not discern whether TGF β 1 alleles were associated with severity in lung disease, malnutrition or both. Regression analysis was used here to quantify the inter-relatedness 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 while 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 FEV1, even among individuals in different environments. Estimates of heritability obtained for cross-sectional FEV1 in various healthy adult populations (0.5 to 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 upon longitudinal measures of lung function in healthy individuals has been published⁵². The aforementioned study used FEV1 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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Figure Legends

Figure 1: Distribution of the cross-sectional measures of lung function in the CF twins and siblings. **A.** The x-axis represents FEV1 expressed as %-predicted based on the equations of Knudson (MaxFEV1%pred), in increments of 10%. The y-axis shows the percentage of study patients with a MaxFEV1%pred that falls within each 10% increment. **B.** FEV1 expressed as CF-specific percentile based on the equations of Kulich (FEV1CF%), in increments of 0.05. The y-axis shows the percentage of study patients with an FEV1CF% that fall within each 0.05 increment. FEV1CF% 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).

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 PFT data while living at home with an affected sibling. The x-axis represents the AvgFEV1CF% and the y-axis represents the EstFEV1%@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).

Figure 3: Correlation of the longitudinal measure of CF-specific lung function in twins and siblings. For each plot, the x-axis represents the AvgFEV1CF% for Twin or Sibling A and the y-

axis represents the AvgFEV1CF% 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.

Table 1. Characteristics of Study Subjects

	Monozygous Twins	Dizygous Twins	Siblings	Combined DZ twins/siblings*
Individuals	94 (47 pairs)	20 (10 pairs)	412 (231 pairs)**	149 (79 pairs) †
Average Age +/- SD at most recent PFT (Range)	20.2 ± 8.2 yrs (7.8–46.7 yr)	17.6 ± 7.1 yrs (10.8-34.5 yrs)	16.8 ± 6.7 yrs (6.8-46.0 yrs)	16.9 ± 6.3 (7.8-39.0 yrs)
Sex				
Female	44	7	190	59
Male	50	13	222	90
Pancreatic Status***				
Insufficient	93	19	368	139
Sufficient	1	1	43	9
Unknown	0	0	1	1
CFTR Genotype				
ΔF508/ΔF508	56 (59.6%)	10 (50%)	203 (49.3%)	75 (50.3%)
Non-ΔF508 Homozygotes	38 (40.4%)	10 (50%)	209 (50.7%)	74 (49.7%)

* Same sex DZ twins and same sex siblings with less than 3 years difference in age

** 14 families with 3 affected children (counted as 3 pairs), 1 family with 4 affected children (counted as 6 pairs).

*** Physician diagnosed pancreatic insufficiency or individual taking supplemental pancreatic enzymes.

† 3 families with 3 affected children (counted as 3 pairs)

Table 2: The Magnitude and Significance of Factors Influencing Longitudinal Measures of Lung Function.

	Coefficient*	95% C.I.**	p-value
EstFEV1%@20yrs			
AvgBMIZ	15.6 ± 1.6	12.5-18.7	<0.001
ΔF508	-3.4 ± 2.2	-7.7-1.0	0.132
Pancreatic Status	11.2 ± 5.8	-0.2-22.7	0.053
Max. Test Age	0.2 ± 0.3	-0.4-0.7	0.550
Constant	90.7 ± 6.0	78.8-102.6	<0.001
Best Fit Model: EstFEV1%@20yrs = 88.4 + (13.8 if pancreatic sufficient) + (15.4 6 x AvgBMIZ)			
AvgFEV1CF%			
AvgBMIZ	0.126 ± 0.013	0.100-0.152	<0.001
ΔF508	0.001 ± 0.019	-0.036-0.038	0.972
Pancreatic Status	0.034 ± 0.051	-0.067-0.134	0.508
Max. Test Age	0.004 ± 0.002	-0.000-0.008	0.074
Constant	0.555 ± 0.048	0.460-0.649	<0.001
Best Fit Model: AvgFEV1CF% = 0.626 + (0.124 x AvgBMIZ)			

* 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.

Table 3: Intra-pair Correlations for Cross-Sectional and Longitudinal Measures of CF Lung Disease Severity in Twins and Siblings Living Together

	Max- FEV1CF% (n)	EstFEV1% @20yrs[†] (n)	Adjusted¹ EstFEV1% @20yrs (n)	AvgFEV1- CF%^{††} (n)	Adjusted² AvgFEV1- CF% (n)
Monozygous Twins	0.88 ± 0.08* (38)	0.81 ± 0.01 (34)*	0.80 ± 0.01 (34)*	0.91 ± 0.01 (36)*	0.93 ± 0.00 (32)*
Dizygous 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 (184)*	0.41 ± 0.01 (90)**	0.43 ± 0.01 (87)*	0.55 ± 0.00 (124)*	0.49 ± 0.00 (117)*
Same Sex Siblings, <3 years difference in age	0.53 ± 0.01 (61)*	0.51 ± 0.03 (37)***	0.36 ± 0.03 (37)	0.65 ± 0.01 (47)*	0.54 ± 0.02 (45)***
Same Sex DZ Twins and Siblings, < 3 years difference in age	0.54 ± 0.03 (67)*	0.50 ± 0.02 (42)**	0.40 ± 0.03 (42)****	0.64 ± 0.07 (52)*	0.58 ± 0.06 (50)*

¹ EstFEV1%@20yrs adjusted for AvgBMIZ and for pancreatic status

² AvgFEV1CF% adjusted for AvgBMIZ

[†] Using minimum of 5 years of PFT data

^{††} Using minimum of 4 years of PFT data

* p-value < 0.0001

** p-value < 0.001

*** p-value < 0.005

**** p-value < 0.01

Table 4: Heritability Estimates for Cross-Sectional and Longitudinal Measures of Lung Function

	Twins and Siblings [†]		All Siblings ^{††} (SE)	
	All Subjects	ΔF508 Homozygotes	All Subjects	ΔF508 Homozygotes
MaxFEV1CF%	0.68	0.86	0.68 (0.14) [*]	0.54 (0.22) ^{**}
AvgFEV1CF%	0.54	0.80	1.00 (no SE) [*]	0.96 (0.19) [*]
AdjAvgFEV1CF%	0.70	0.78	0.96 (0.14) [*]	0.89 (0.17) [*]
EstFEV1%@20yrs	0.62	0.56	0.73 (0.19) ^{**}	0.65 (0.28)
AdjEstFEV1%@20yrs	0.82	0.76	0.83 (0.20) ^{**}	1.00 (no SE) [*]

[†] 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 age³⁰

^{††} 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; <http://www.sfbr.org/solar>)³¹.

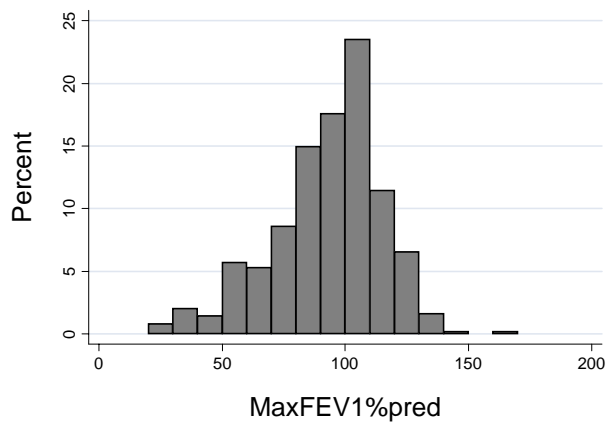
Standard errors for each estimate are shown in parentheses.

^{*} p-value < 0.0001

^{**} p-value < 0.001

Figure 1:

A.



B.

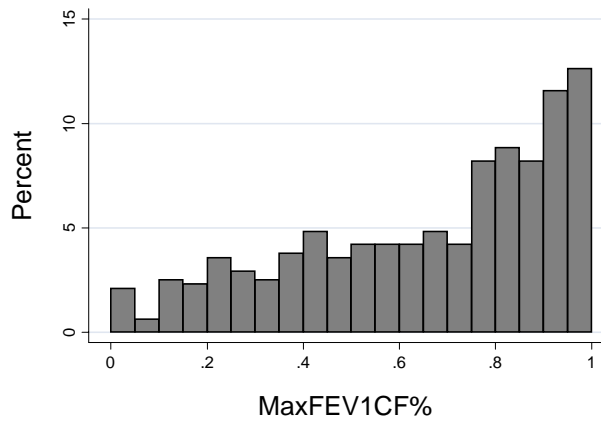


Figure 2A:

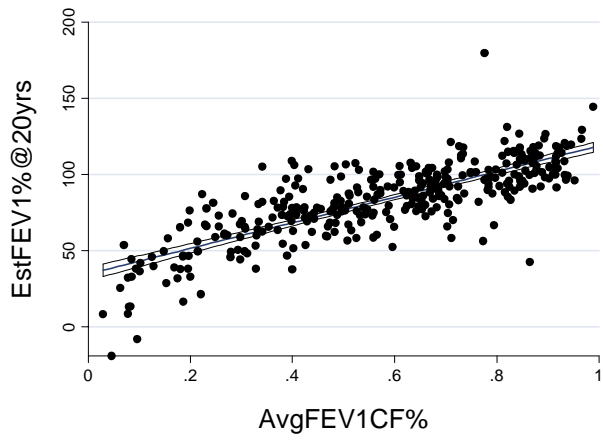


Figure 2B:

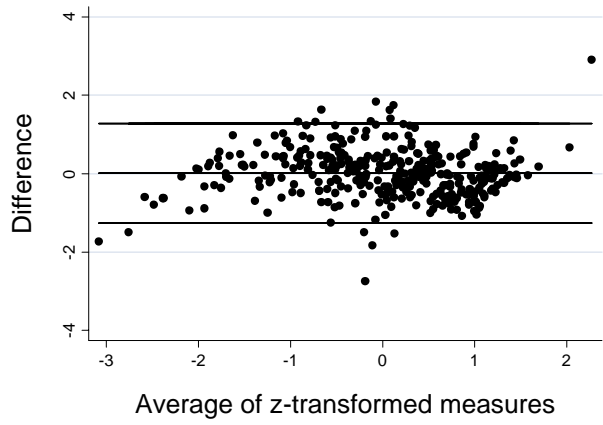
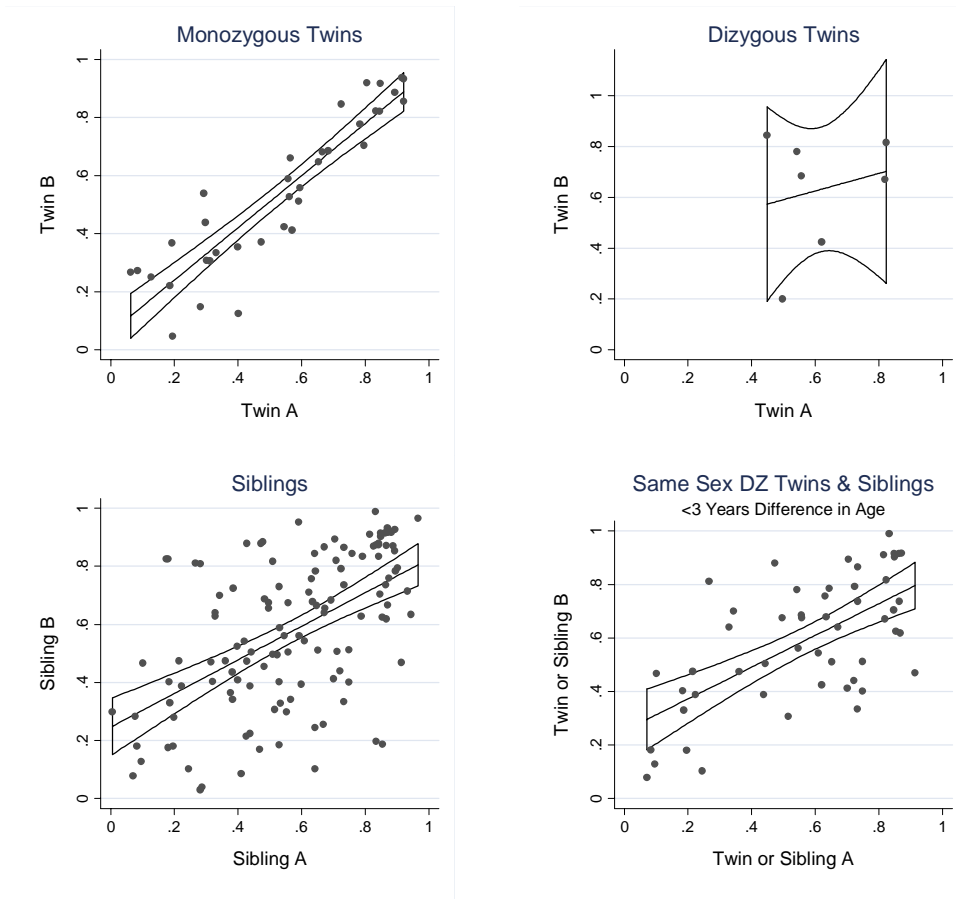


Figure 3:



Online Data Supplement

Heritability of Lung Disease Severity in Cystic Fibrosis

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Table E1: Intra-pair Correlations for Cross-sectional and Longitudinal Measures of CF Lung Disease Severity for Twins and Siblings Less than 15 Years of Age and Greater than 15 Years of Age, Living Together

	MaxFEV1CF%	AvgFEV1CF%	EstFEV1%@20yrs
MZ Twins, Both <15 Years of Age	0.90 (14) [*]	0.94 (8) ^{**}	0.84 (7) ^{***}
MZ Twins, Both >15 Years of Age	0.88 (22) [*]	0.89 (25) [*]	0.85 (24) [*]
Same Sex DZ Twins and Siblings, Both <15 Years of Age	0.55 (46) ^{**}	0.67 (31) [*]	0.51 (24) ^{***}
Same Sex DZ Twins and Siblings, Both >15 Years of Age	0.53 (16) ^{***}	0.61 (16) ^{***}	0.54 (15) ^{***}

* p-value < 0.0001

** p-value < 0.001

*** p-value < 0.05

Table E2: Correlations for Cross-Sectional and Longitudinal Measures of CF Lung Disease Severity in Δ F508 Homozygous Twins and Siblings Living Together at Home

	Max- FEV1CF% (n)	EstFEV1% @20yrs[†] (n)	Adjusted¹ EstFEV1% @20yrs (n)	AvgFEV1- CF%^{††} (n)	Adjusted² AvgFEV1- CF% (n)
Monozygous Twins	0.92 ± 0.01 (23) [*]	0.86 ± 0.02 (22) [*]	0.82 ± 0.02 (22) [*]	0.93 ± 0.01 (22) [*]	0.90 ± 0.01 (20) [*]
Dizygous Twins	0.59 ± 0.13 (4)	-0.18 ± 0.60 (3)	0.61 ± 0.24 (3)	0.40 ± 0.37 (3)	0.75 ± 0.10 (3)
Siblings	0.28 ± 0.01 (93) ^{****}	0.36 ± 0.02 (48)	0.46 ± 0.02 (48) ^{****}	0.41 ± 0.01 (67) ^{**}	0.38 ± 0.01 (64) ^{***}
Same Sex Siblings, <3 years difference in age	0.50 ± 0.02 (31) ^{****}	0.59 ± 0.03 (21) ^{****}	0.45 ± 0.04 (21)	0.54 ± 0.02 (26) ^{***}	0.51 ± 0.02 (25) ^{**}
Same Sex DZ Twins and Siblings, < 3 years difference in age	0.49 ± 0.02 (34) ^{****}	0.58 ± 0.03 (23) ^{****}	0.44 ± 0.03 (23)	0.53 ± 0.02 (28) ^{***}	0.51 ± 0.02 (27) ^{****}

¹ EstFEV1%@20yrs adjusted for AvgBMIZ and for pancreatic status

² AvgFEV1CF% adjusted for AvgBMIZ

[†] Using minimum of 5 years of PFT data

^{††} Using minimum of 4 years of PFT data

^{*} p-value < 0.0001

^{**} p-value < 0.001

*** p-value < 0.005

**** p-value < 0.01