

Genetic modifiers play a substantial role in diabetes complicating cystic fibrosis*

Scott M. Blackman, M.D., Ph.D.^{1,2}, Stephanie Hsu, M.D., Ph.D.¹,

Lori L. Vanscoy, M.D.³, J. Michael Collaco, M.D.^{2,4}, Sarah E. Ritter, B.A.²,

Kathleen Naughton, M.S.², Garry R. Cutting, M.D.²

Author affiliations: ¹Division of Pediatric Endocrinology, Johns Hopkins University School of Medicine, Baltimore, MD and ²McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, ³Department of Pediatrics, National Naval Medical Center, Bethesda, MD, ⁴Pediatric Respiratory Sciences, Johns Hopkins University, Baltimore, MD.

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Corresponding author and reprint requests to: Dr. Garry R. Cutting, M.D.

Mailing address: McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, 733 N. Broadway, Broadway Research Building 559, Baltimore, MD 21205

Phone: (410) 614-0212

Fax: (410) 614-0213

E-mail: gcutting@jhmi.edu

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ABSTRACT

Context: Insulin-requiring diabetes, affects 7-15% of teens and young adults and >25% of older adults with cystic fibrosis (CF). Pancreatic exocrine disease caused by *CFTR* dysfunction underlies the high rate of diabetes in CF patients; however, only a subset develops this complication, indicating that other factors are necessary.

Objective: To estimate the relative contribution of genetic and non-genetic modifiers to the development of diabetes in CF.

Design/Patients: Twin and sibling study involving 1366 individuals at 109 centers in the CF Twin and Sibling Study (68 monozygous twin pairs, 23 dizygous twin pairs, and 588 sibling pairs, all with CF).

Main Outcome Measure: Chronic, insulin-requiring diabetes in the setting of CF, as established using longitudinal clinical and biochemical data.

Results: About 9% of this predominantly pediatric population (mean age = 15.8 years) had diabetes. Key independent risk factors identified by regression modeling included having a twin or sibling with CF and diabetes, increasing age, pancreatic exocrine insufficiency or two mutations causing severe *CFTR* dysfunction, decreased lung function or decreased body mass index, and longer duration of glucocorticoid treatment. The concordance rate for diabetes was substantially higher in monozygous twins (0.73) than in dizygous twins and siblings with CF (0.18; $p=0.002$). Heritability was estimated as near 1 (95% CI, 0.42 to 1.0).

Conclusions: Diabetes is a frequent complication of CF that is associated with worse outcomes. While a non-genetic factor (steroid treatment) contributes to risk, genetic modifiers (i.e. genes other than *CFTR*) are the primary cause of diabetes in CF.

INTRODUCTION

Cystic fibrosis was first recognized as a disorder of malnutrition due to pancreatic exocrine deficiency, and the development of pancreatic enzyme replacement therapy was accompanied by dramatic improvement in survival from infancy to adolescence (1). Obstructive lung disease is currently the major cause of morbidity and mortality for CF patients, and pulmonary therapies have helped increase median life expectancy to 36 years for a newborn with CF (2). The discovery that CF is caused by dysfunction of an epithelial chloride channel, the cystic fibrosis transmembrane conductance regulator (*CFTR*), provided a unifying molecular explanation for disturbances of electrolyte and fluid transport in the lungs, pancreas and sweat gland (3). These successes have resulted in a larger fraction of CF patients entering into adulthood with the concomitant realization that diabetes is a frequent complication of this disorder (4).

While diabetes affects few children with CF, its prevalence steadily increases in adolescence and adulthood, with at least 25% of those over 20 being affected (reviewed in (5)). Diabetes in CF has features of both type 1 and type 2 diabetes seen in the general population but has generally been considered to be a distinct entity (6). Diabetes in CF patients typically occurs in the absence of obesity and is associated with a significantly worse prognosis (7;8). Treatment of diabetes improves nutritional status and pulmonary function (9;10). Elucidating the mechanisms underlying this increasingly prevalent complication is essential to continued improvement in the survival of CF patients and may shed light on similar conditions in the general population. The *CFTR* gene itself plays a role, as CF patients with mutations causing mild *CFTR* dysfunction are not at increased risk for diabetes (6). Also, it has been suggested that other *CFTR* mutations may confer differential risk for diabetes (11;12) although these observations have not been replicated to date. However, other factors must be involved, as diabetes affects only a fraction of patients with identical *CFTR* genotypes (e.g., (13)). Although twin and other studies have established that type 1 and type 2 diabetes have a strong genetic component (reviewed in (14)), the extent to which genes (other than *CFTR*) and environmental factors modulate diabetes risk in

CF patients is unknown. In the current study of affected twins and siblings, we derived the contribution of genes to the development of diabetes in CF patients.

MATERIALS AND METHODS

Study subjects. Clinical data and DNA samples were collected by the Cystic Fibrosis Twin and Sibling Study (15;16), with additional clinical data provided by the U.S. CF Foundation Patient Registry (Bethesda, MD). Enrollment was based on a diagnosis of CF (17) and having an affected twin and/or sibling. Informed consent was obtained from all subjects in the study. Isolation of patient DNA, identification of *CFTR* mutations and zygosity testing have been previously described (15).

Phenotype definitions. Recognizing that waxing and waning hyperglycemia in CF can lead to ambiguity in a diagnosis of diabetes, we used longitudinal data to identify patients with chronic, insulin requiring diabetes as well as those with clearly normal glucose metabolism. Clinical information (e.g. age or date of diabetes diagnosis, clinician diagnoses, treatment modality, and diagnostic testing obtained as part of clinical management) was obtained from the medical record and from the CF Foundation (CFF) Patient Registry. Fasting hyperglycemia (≥ 126 mg/dl or 7 mM) as recorded in the CFF Registry was found to be an inconsistent indicator of diabetes after review of clinic notes for the 13 patients in question, so this particular parameter was not used to classify patients. The CFF Registry specifies for each encounter and/or at year's end whether a patient had diabetes (or was euglycemic, starting with 2006 data), was treated chronically or intermittently with insulin, treated with oral agents, or with diet. Patients were classified as having Diabetes, Possible Diabetes, Hyperglycemia, and being Euglycemic by the scheme shown in Figure 1.

Patient age was as of the most recent clinical data. Steroid treatment was defined as use of systemic (i.e., oral or intravenous) glucocorticoids, excluding intranasal and inhaled steroids. Duration of steroid treatment and diagnosis of allergic bronchopulmonary aspergillosis (ABPA) were defined by CF clinicians. Other phenotype definitions (e.g., pancreatic insufficiency, measures of lung function,

MaxFEV1CF% and AvgFEV1CF% (16) and nutritional status, body mass index standard deviation score (18)) are described elsewhere (15).

Statistical Analysis. Statistical calculations were performed using Intercooled Stata 10 (StataCorp, College Station, TX). Heritability was estimated by the liability-threshold model (19) and by logistic regression (20). The logistic regression approach of Ramakrishnan et al. (20) was extended to the use of Cox regression for censored phenotypes (e.g., age of diabetes diagnosis). A p-value of less than 0.05 was considered statistically significant. In case of small cell sizes, the Fisher exact test was used (<http://www.psych.ku.edu/preacher/fisher/fisher.htm>). The Student t test was used to compare normally distributed continuous data.

RESULTS

A substantial fraction of patients in the CF Twin and Sibling study have abnormal glucose metabolism.

Clinical information was collected for 1370 CF-affected individuals and 883 parents in 679 families in the CF Twin and Sibling Study. Four patients were excluded for antibody-positive type 1 diabetes. Based on diagnostic and laboratory information in the medical record and CF Foundation Patient Registry, the remaining 1366 patients were classified as Euglycemic (n=712), Hyperglycemic (n=415), Possible Diabetes (n=52), Diabetes (n=128), or had insufficient information to be classified (Unknown; n=59) (Figure 1).

There was general agreement between severity of diagnostic category and clinical parameters such as the patient's stated diagnosis or their oral glucose tolerance test results (Table 1). Of the 128 patients categorized as having Diabetes, 77 patients (60%) had at least two glucose tolerance tests in the diabetic range (fasting plasma glucose ≥ 126 mg/dl / 7 mM or 2-h glucose ≥ 200 mg/dl / 11.1 mM) or one hemoglobin A1c measurement of 7% or greater, and 97 (76%) had at least one glucose measurement in the diabetic range. Most of those classified as Diabetes without confirmatory glucose data were labeled as not having been tested due to already having diabetes; others included one patient with one recorded glucose measurement despite 7.5 years of daily insulin treatment, and four chronically insulin-treated patients with glucose data available only prior to diabetes diagnosis. The distribution of diabetes, impaired glucose tolerance (using Hyperglycemia or Possible Diabetes as a proxy), and normal glucose tolerance (using Euglycemia as a proxy) in this predominantly pediatric study group (mean age 15.8 years) is comparable to that reported in other retrospective (2;13) and prospective (21-24) reports.

Risk factors for Diabetes include pancreatic insufficiency, reduced lung function, steroid treatment, and diabetes in a CF-affected sibling.

Clinical characteristics of the Twin and Sibling study patients (Table 2) demonstrate that risk factors for diabetes in the Twin and Sibling study are similar to what has been reported by others for unrelated CF patients. Diabetes was associated with increasing age, exocrine pancreatic insufficiency, and two “severe” *CFTR* mutations (e.g., nonsense or frameshift), reduced lung function, reduced body mass index, steroid use, and ABPA (13;25). The same risk factors were apparent either by comparing Diabetic with Euglycemic patients (e.g., Fisher exact or t-test), or by comparing Diabetic with all other patients (odds ratios by logistic regression). Age, sex, and pancreatic insufficiency rate differed between Euglycemic and Diabetes groups, so we derived a subset of Euglycemic patients who were matched to the Diabetes patients for the three variables (Matched Euglycemic). After matching, all variables except *CFTR* genotype (which is highly associated with pancreatic insufficiency (26)) and longitudinal nutritional status remained different.

Multivariate regression was used to examine interaction between risk factors. Confounding among correlated variables was evident when all factors were included (Model 1 in Table 3). Subsequent models were built both by stepwise addition of risk factors (keeping those that were significant), and by stepwise removal of the least significant risk factors. Patient age, pancreatic exocrine insufficiency, and having a twin or sibling with Diabetes were found to be independent risk factors in all models. Sets of variables that were found to confound each other in multivariate models included: pancreatic exocrine insufficiency and having 2 severe *CFTR* mutations; cross-sectional and longitudinal lung function; all 4 measures of lung function and nutritional status, and steroid treatment and ABPA. Correlations among these sets of variables have been reported previously (16;26;27). In the second model shown in Table 3, we retained the member of each set of confounding variables with the greatest amount of available information. Female sex, identified by some as a risk factor for diabetes (discussed in (24)), was not a statistically significant risk factor in this population, though the multivariate odds ratio of 1.6 in the

second model was of borderline significance ($p=0.052$). In summary, interactions among risk factors for diabetes in twins and siblings with CF are similar to those identified in unrelated CF patients.

Steroid treatment induces transient hyperglycemia and diabetes in CF (28) (though not in all studies (8;13)), and duration of steroid treatment was found to be a quantitative risk factor for diabetes in this study. Therefore, we assessed different lengths of treatment to determine the interval with the highest effect on diabetes risk in our study. Defining steroid treatment as any duration, for more than 7 days, or for more than 15 days (in the year prior to enrollment) were less potent risk factors and significant only in univariate regression. Steroid treatment for a minimum of either 30 or 60 days' duration was a significant risk factor with similar odds ratios in all analyses (not shown). Therefore, we defined "prolonged" exposure as more than 30 days of steroid treatment in the year prior to enrollment.

Of note, relatedness (ie. having a twin or sibling with Diabetes) was found to be a substantial risk factor in all comparisons (Table 2). Even after adjusting for age, sex, pancreatic exocrine insufficiency, lung function, and prolonged steroid treatment, the odds ratio of a CF patient with diabetes having a monozygous (MZ) twin with diabetes was 31.9 ($p<0.001$, 95% CI = 9.7-105), while the odds ratio for having a dizygous (DZ) twin or sibling with diabetes was 3.5 ($p<0.001$, 95% CI = 1.8-7.0). The odds ratios differed from each other ($p<0.001$). Furthermore, Cox regression using age of diagnosis of diabetes as the phenotype and censoring at the current age (not shown) also demonstrated that having a MZ twin or DZ twin/sibling with diabetes each conferred risks for diabetes that were different from each other (MZ twin with diabetes, hazard ratio (HR) = 3.6; DZ twin or sibling with diabetes, HR = 2.0; $p<0.001$).

Concordance for diabetes correlates with the degree of gene sharing between CF patients.

To quantify the contribution of genes other than *CFTR* to diabetes in CF, we determined concordance rates (i.e., how often both members of a pair have disease divided by the number of pairs where at least one member has disease) in 68 pairs of MZ twins, 23 pairs of DZ twins, and 588 pairs of siblings with CF. Concordance for diabetes was 0.73 (11 of 15 pairs) in MZ twin pairs (Table 4),

indicating the importance of shared genes and/or shared environment. The difference in concordance rates between MZ twins, sharing 100% of genes, and DZ twins, sharing 50% of genes on average, estimates the proportion of disease risk that can be attributed to genes (i.e. heritability) for diabetes (29). Because the few DZ twin pairs were too young, on average, to be at risk for diabetes, limited conclusions could be drawn from this group. Therefore, affected sibling pairs (who share 50% of genes on average, like DZ twins) were analyzed for concordance for diabetes. To account for the possibility that concordance was decreased in siblings due to differing age and sex (which could have led to the assignment of environmental effects as genetic), we selected same-sex sibling pairs and considered the diabetes status of the older sibling when he/she was the age of the younger sibling. To account for the dependence of diabetes prevalence on age (and possibly cohort), we selected a subset of siblings matched to the MZ twins for age, sex, and pancreatic insufficiency. Concordance for diabetes among the age and sex matched siblings was 0.18 (4 of 22 pairs; Table 4) which significantly differed from the concordance rate in MZ twins (0.73, $p=0.002$) and yields heritability for diabetes in CF estimated at 0.98 (95% CI, 0.42 to 1.0).

To account for the possibility that matched siblings were a biased sample, we evaluated the entire sibling group. Thirteen of 82 (16%) CF sibling pairs had both members affected with diabetes, significantly different from MZ twins ($p=1.9\times 10^{-5}$). Similar concordance rates were obtained when correcting only the diabetes status for differing ages of siblings (8 of 61; 0.13), when selecting same-sex pairs only (7 of 44; 0.16) or when doing both (6 of 30; 0.20). Alternatively, sibling pairs were corrected for age by considering pairs differing by less than 3 years of age, also yielding a low concordance rate (5 of 44; 0.12). Thus, the wide gap in concordance between MZ twins and siblings could not be attributed to bias introduced by the matching process.

To assess whether the restrictive definition for diabetes strongly influenced the heritability estimate, a second analysis was performed, in which those in either the Possible Diabetes or Diabetes categories were considered to be affected with diabetes. Heritability remained high but was slightly decreased (MZ concordance = 13 of 20, 0.65; matched DZ+Sibling concordance = 9 of 36, 0.25;

heritability = 0.8). Thus, the estimated effect of genetic modifiers on the development of diabetes in CF remained high even when using a less restrictive definition of diabetes.

Factors correlated with diabetes in CF do not affect the estimate of genetic effect

Diabetes was correlated with other factors (e.g., pancreatic exocrine insufficiency, steroid treatment, and lung function) which, if influenced by genetic factors, could inflate the heritability estimate for diabetes. However, estimates of genetic effect (independent of the *CFTR* gene) were low for both pancreatic insufficiency ($h^2=0$) and steroid treatment ≥ 30 days ($h^2=0.3$) (data not shown). Neither result is unexpected, as pancreatic exocrine insufficiency is known to be determined primarily by *CFTR* genotype (26), and steroid treatment is an environmental factor. Furthermore, when considering only twins and siblings who both had exocrine pancreatic insufficiency, essentially identical concordance rates for diabetes were derived (data not shown). The same was true for twins and siblings who were homozygous for the common CF mutation, $\Delta F508$, or who had two mutations causing severe *CFTR* dysfunction (data not shown). Likewise, accounting for systemic steroid treatment (≥ 30 days) had little effect on concordance rates which remained markedly different between MZ twins and DZ twins + Siblings (Table 4). Although ABPA, a severe form of asthma treated with steroids, was not an independent risk factor in this regression analysis, others have found ABPA to be associated with diabetes (e.g., (13)). Adjustment for ABPA had a negligible effect on concordance rates for diabetes (Table 4). Diabetes was also correlated with lung function, itself a heritable phenotype (16). To test whether this interaction inflated the estimate of diabetes heritability, concordance rates were compared between 64 MZ twin pairs and 128 sibling pairs matched both for pair averages of cross-sectional (MaxFEVCF%) and longitudinal (AvgFEVCF%; see Methods section for definitions) measures of CF lung function (to enable comparison between MZ and sibling pairs) and for intra-pair differences in these phenotype measures (so that MZ and matched sibling pairs have similar degrees of concordance for lung function). No significant differences were seen in matched variables ($p=0.2-0.9$). Concordance rates for diabetes were essentially unchanged

after accounting for lung function (0.73 (11 of 15) in MZ twins compared to 0.21 (4 of 19) in sibling pairs; $p=0.005$, Fisher exact). Thus, these important risk factors for diabetes in CF do not confound the estimated genetic contribution to diabetes risk.

To estimate genetic effect while simultaneously accounting for multiple covariates, heritability was estimated by regression analysis (20). Using diabetes as a dichotomous variable, multivariate logistic regression (including age and sex as covariates) estimated heritability as approaching 1.0 (0.99; 95% CI 0.98-1.0). Extending the regression approach to the use of a time-censored variable (age of diabetes diagnosis), multivariate Cox regression (with the same covariates) yielded similarly high heritability estimates for age of onset of diabetes (0.99; 95% CI 0.92-1.0). Essentially identical results were obtained when including prolonged steroid treatment and cross-sectional or longitudinal lung function in the regression model (not shown). Thus, the high heritability estimate for diabetes in CF could not be attributed to correlation with other traits that are under genetic control.

DISCUSSION

Pancreatic exocrine insufficiency (which is highly correlated with specific *CFTR* mutations (26)) was a near-universal prerequisite for developing diabetes in this study. However, not all patients with pancreatic insufficiency develop this complication. We demonstrate here that the risk of diabetes correlates with the degree of gene sharing among related CF patients indicating that genes other than *CFTR* contribute substantially to diabetes complicating CF. Three analyses were performed to quantify the effect of these genetic modifiers of diabetes in CF. First, multivariate analysis revealed that having a monozygous (MZ) CF twin with diabetes (100% gene sharing) independently conferred a high risk of diabetes (similar to that of pancreatic insufficiency) and that having a sibling or dizygous (DZ) twin with diabetes (50% gene sharing) also conferred a significant, though, lower risk. Second, comparison of MZ twin pairs and age- and sex-matched DZ twins and siblings revealed that MZ twins had substantially higher rates of concordance for diabetes. Third, accounting for important diabetes risk factors by

matching or by regression had negligible effect on the estimate of a strong genetic component (i.e. heritability) for diabetes risk in CF.

A major strength of this study is that recruitment of subjects in the CF Twin and Sibling Study was based on a diagnosis of CF and not diabetes. Thus, biases of ascertainment or phenotype definition (when the second member of a pair receives greater scrutiny for the diagnosis of diabetes) were minimized. Furthermore, the high density of clinical data available (standard of care is to see CF patients every 3 months) allowed for detailed characterization of diabetes phenotype in a longitudinal manner, making it possible to distinguish between intermittent and chronic diabetes. Weaknesses of the study included the small number and young age of dizygous twins that necessitated the use of siblings as proxies for individuals sharing 50% of their genes. However, the high estimate of heritability is primarily due to the high MZ twin concordance rate. This study was not able to distinguish between the effects of shared genes and shared intrauterine environment (or features thereof, e.g., the placenta), which is a recognized limitation even of classic twin studies (25). The absence of concordant DZ twin pairs suggests that such unaccounted sources of between-sibling variability are not likely to affect the conclusion that genetic modifiers are largely responsible for diabetes in CF. Also, we did not have complete retrospective data for fasting glycemia which would have allowed classifying patients strictly by CF consensus criteria (6). However, treatment with insulin or oral agents is a reasonable proxy for fasting hyperglycemia for patients treated according to those same guidelines. Furthermore, the insidious onset of diabetes in CF can lead to misdiagnoses, especially if only a single point in time is considered. Our use of longitudinal data should have reduced misclassification of Euglycemic patients by requiring multiple normal screening tests. These data also allowed us to distinguish those with intermittent hyperglycemic episodes (e.g., during disease exacerbations and/or treatment with systemic steroids) from those with chronic diabetes. Finally, our criteria may have placed some CF patients with chronic diabetes in the Hyperglycemic or Possible Diabetes categories. However, this approach should have minimized the false

positive classifications in the Euglycemic and Diabetes categories, albeit at the cost of reducing study power.

While genetic modifiers are an important cause of diabetes in CF, non-genetic factors (such as treatment with steroids) also play a role. There is significant agreement between risk factors found in the CF families in the Twin and Sibling study and what has been seen for unrelated CF patients. As noted here, diabetes has been associated with increasing age, pancreatic insufficiency, reduced lung function, poor nutritional status, and ABPA and/or systemic steroid treatment (13;25;30). We found that increasing duration of steroid treatment and prolonged steroid treatment (over a threshold of 30 days) were more potent risk factors for diabetes in CF than steroid treatment of no specific duration. Treatment duration may be a proxy for cumulative steroid dose, which has been reported to be a risk factor for steroid-induced diabetes in the general population (31). Female sex has been noted to be a significant risk factor for diabetes in CF, and we saw evidence of this association in this study, although significance was not achieved which may be due to insufficient power (13). Other associated CF complications such as lung transplantation (32), markers of chronic inflammation (24), and liver disease (33) were not evaluated.

Results of this study enable a comparison of genetic contribution to diabetes in CF to type 1 and type 2 diabetes in the general population. The concordance rate of diabetes among CF siblings (0.18) divided by the frequency of diabetes in this family study (0.09) estimates the sibling recurrence risk ratio at 2.0. When adjusting for pertinent covariates, the sibling odds ratio was estimated at 4.6 (logistic regression) and 2.0 (hazard ratio; Cox regression). Of note, the estimates of sibling recurrence risk for diabetes in CF patients are more comparable to recent estimates for type 2 diabetes (sibling recurrence risk ranging from 1.2 to 1.8 (34)) than to type 1 diabetes (sibling recurrence risk of about 15 (35)) while heritability estimates are higher than both type 1 and type 2 diabetes (0.72-0.88 (36) and 0.26 to 0.61 (37), respectively). Thus, diabetes complicating CF demonstrates a degree of genetic control at least as high as other forms of diabetes occurring in the general population.

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FIGURE LEGENDS

Figure 1. Diabetes diagnostic flowchart. Selection processes: 1: Diabetes classification required a diagnosis of diabetes and treatment with insulin or oral agents for at least 1 year; 2: Possible Diabetes required any of: intermittent or previous diabetes and treatment (n=34), current diabetes but no treatment (n=15), glucose tolerance in diabetic range (6) (n=2) or hemoglobin A1c > 7% (n=1) but no diabetes diagnosis and no treatment; 3: Hyperglycemia classification required any of: diagnosis of “impaired glucose tolerance,” diabetes which resolved without treatment, insulin treatment without diabetes, impaired glucose tolerance or impaired fasting glucose by ADA criteria (38), or hemoglobin A1c above the reference range (> 6%); 4: Euglycemia classification required diagnosis of “Normal,” at least 2 glucose measurements available, and all glucose and hemoglobin A1c levels within normal limits. Classification as Unknown included those with unknown diabetes diagnosis, unknown treatment, or fewer than two glucose measurements. “Treatment” refers to insulin or oral medication but does not include dietary treatment.

TABLES

Table 1. Glucose metabolism profiles of 1366 study subjects

	Euglycemic (n=712)	Hyperglycemic (n=415)	Possible Diabetes (n=52)	Diabetes (n=128)	Unknown (n=59)
Reported Diagnosis*	712 normal	327 normal 88 IGT	2 normal 1 IGT 34 intermittent DM 15 diabetes	128 diabetes	59 none
Use of insulin or oral hypoglycemic agent†	712 none	411 none 2 intermittent 2 chronic	18 none 34 intermittent	128 chronic	59 none
Glucose / A1c criteria‡	712 normal	15 normal 392 IGT 7 DM 1 unknown	3 normal 32 IGT 17 DM	4 normal 46 IGT 77 DM 1 unknown	59 unknown
Ever had fasting glucose \geq 126 mg/dl (7 mM)	0/153	4/202 (2%)	4/32 (12%)	5/64 (8%)	0/4
Ever had random or 2h OGTT > 200 mg/dl (11.1 mM)	0/710	93/414 (22%)	34/52 (65%)	97/127 (76%)	0/59
# glucose tests (mean \pm S.D.)	6.0 \pm 2.4 (n=683)	8.7 \pm 4.8 (n=412)	11.4 \pm 7.9 (n=48)	10.0 \pm 7.2 (n=123)	0.8 \pm 0.4 (n=46)
# tests in diabetic range (mean \pm S.D.)	0	0.2 \pm 0.6	0.8 \pm 1.0	2.0 \pm 2.7	0

*As reported in medical records, including “normal” (normal glycemia), “IGT” (representing diagnoses of impaired glucose tolerance, impaired fasting glucose, or hyperglycemia), “intermittent DM” (intermittent or transient diabetes), or diabetes.

†Diabetes medication treatment as grouped into none, intermittent, or chronic (>1 yr).

‡Classified as normal, impaired glucose tolerance (IGT), diabetes (DM), or unknown based on glucose testing data interpreted by ADA criteria (38), and hemoglobin A1c data (normal < 6%; diabetes \geq 7%; see Fig. 1).

Table 2. Comparison of clinical features of CF patients in the twin/sibling study group with Euglycemia, Hyperglycemia or Possible Diabetes, or Diabetes

	Euglycemic	Hyperglycemia or Possible Diabetes	Diabetes	Odds ratio*	Matched† Euglycemic	Odds ratio†
Number of subjects	712	467	128		128	
Mean age (years)	13.6‡	16.9‡	24.2	1.08 per year (n=1307)‡	24.3	1.0 (n=256)
Female	345/712 (48%)	212/467 (45%)	67/128 (52%)	1.2 (n=1307)	67/128 (52%)	1.0 (n=256)
CFTR genotype: ΔF508 ×2§	329/702 (47%)	237/465 (51%)¶	77/126 (61%)	1.7 (n=1293)	82/126 (65%)	0.8 (n=252)
Severe CFTR ×2#	514/661 (78%)‡	393/434 (91%)	112/118 (96%)	3.9 (n=1213)	118/124 (95%)	0.9 (n=242)
Pancreatic Insufficiency	534/619 (86%)‡	406/429 (95%)¶	115/116 (99%)	13.9 (n=1164)	120/120 (100%)	n/a
Lung function Percentile: **						
Cross-sectional	0.74 (n=564)‡	0.66 (n=429)‡	0.55 (n=124)	0.81 per 10% (n=1117)‡	0.74 (n=127) ‡	0.78 per 10% (n=251)‡
Longitudinal	0.65 (n=368)‡	0.58 (n=355)	0.51 (n=117)	0.83 per 10% (n=840)‡	0.64 (n=123)‡	0.80 per 10% (n=240)‡
Nutritional status: ††						
Cross-sectional	-0.04 (n=695)‡	-0.46 (n=467)	-0.74 (n=126)	0.68 per SD unit (n=1288)‡	-0.31 (n=128)	0.7 per SD unit (n=254)
Longitudinal	-0.01 (n=666)‡	-0.30 (n=452)¶	-0.51 (n=119)	0.61 per SD unit (n=1237)‡	-0.36 (n=123)	0.8 per SD unit (n=242)
Steroid treatment (≥30 days) ††	133/712 (19%)‡	105/467 (22%)‡	58/120 (45%)	3.3 (n=1307)‡	23/128 (18%)‡	3.8 (n=256)‡
Steroid treatment (days/yr) ††	17 (n=416)‡	32 (n=310)‡	87 (n=74)	1.2 per month (n=800)‡	23 (n=84) ‡	1.3 per month (n=158)‡
ABPA ††	29/587 (5%)‡	42/401 (10%)‡	26/105 (25%)	4.3 (n=1093)‡	8/108 (7%)‡	4.1 (n=213)‡
Has an MZ twin with Diabetes	3/71 (4.2%)‡	1/43 (2.3%)‡	21/26 (81%)	57.7 (n=1307)‡	1/25 (4%)‡	25 (n=256)
Has a DZ twin or sibling with Diabetes	22/606 (3.6%)‡	26/410 (6.3%)‡	21/94 (22%)	4.6 (n=1307)‡	16/101 (16%)	1.4 (n=256)

*Odds ratio for patients with Diabetes vs. those in all other groups (Euglycemic, Hyperglycemic, and Possible Diabetes) pooled.

†Matched Euglycemic subjects were selected to match Diabetic subjects for age, sex, and pancreatic insufficiency/sufficiency. Odds ratio is for patients with Diabetes vs. Matched Euglycemic subjects.

‡P<0.001 vs. Diabetes

§Homozygosity for the $\Delta F508$ *CFTR* mutation.

||P<0.01 vs. Diabetes

¶P<0.05 vs. Diabetes

#Both *CFTR* alleles with severe (e.g., frameshift or nonsense) mutations (list available upon request).

**Cross-sectional and longitudinal measures of lung-function were based on forced expiratory volume in 1 second as defined previously (16).

††Nutritional status was assayed by body mass index standard deviation score (BMI SDS), with longitudinal averages as defined previously (18). Cross-sectional BMI SDS was calculated by averaging over the year prior to enrollment.

‡‡Oral or intravenous steroid use and diagnosis of ABPA were reported by either questionnaire or CFF Patient Registry (intranasal and inhaled steroid use were excluded).

Table 3. Independent risk factors for Diabetes identified by logistic regression analyses.

	Model 1 Multivariate OR (n=671)	95% CI	Model 2 Multivariate OR (n=996)	95% CI
Age at last diabetes screen	1.1 per year*	1.08-1.18	1.1 per year*	1.07-1.13
Female	1.4 (NS)	0.84-2.5	1.6 (NS)	1.0-2.6
Pancreatic Insufficiency	8.2‡	1.06-64	23.5*	3.5-160
Lung function Percentile	0.99 per 10% (NS)	0.81-1.2	0.84 per 10%*	0.78-0.91
Longitudinal	0.78 per 10%‡	0.62-0.99	Not in model	
Nutritional status	0.84 per SD unit (NS)	0.6-1.2	Not in model	
Longitudinal	1.5 per SD unit (NS)	0.9-2.5	Not in model	
Steroid treatment (≥30 days)	2.4†	1.2-4.5	2.5*	1.4-4.2
ABPA	1.5 (NS)	0.7-3.0	Not in model	
MZ twin with Diabetes	37.7*	7.6-187	31.9*	9.7-105
DZ twin or sibling with Diabetes	3.1†	1.5-6.4	3.5*	1.8-7.0

*P<0.001

†P<0.01

‡P<0.05

‡Pancreatic insufficiency, two severe CFTR mutations, and two $\Delta F508$ mutations were risk factors for Diabetes when individually included in multivariate models. Together, any two were correlated and confounded each other. The strongest and most significant correlation was with pancreatic insufficiency, which is included in the model shown.

§These measures of lung function and of nutritional status were risk factors for Diabetes when individually included in multivariate models. Together, any two were correlated and confounded each other. The strongest and most-significant correlation was with cross-sectional lung function which is included in the model shown.

||Increasing duration of steroid treatment, presence of prolonged steroid treatment (30 days or more), and presence of ABPA were risk factors for Diabetes when individually included in multivariate models.

Together, any two were correlated and confounded each other. The strongest and most significant correlation was with presence of prolonged steroid treatment, which is included in the model shown above.

Table 4: Diabetes concordance rates in pairs of MZ twins, DZ twins and siblings with CF*

	Median age (yr)	Diabetes status of pair			Concordance [†]
		Both	One	Neither	
MZ twin pairs	18.3 (n=68)	11	4	53	0.73
DZ twin pairs	11.7 (n=23)	0	3	20	0.00
Sibling pairs	13.7 (n=588)	13	69	506	0.16
DZ and Sibling matched to MZ[‡]	17.3 (n=136)	4	18	114	0.18
Diabetes concordance rates after accounting for steroid use ≥ 30 days:[§]					
MZ twin pairs	18.3 (n=66)	8	1	47	0.89
DZ and Sibling pairs	13.4 (n=579)	6	47	430	0.11
DZ and Sibling matched to MZ[‡]	17.1 (n=128)	2	13	90	0.13
Diabetes concordance rates after accounting for ABPA:					
MZ twin pairs	17.9 (n=57)	8	1	48	0.89
DZ and Sibling pairs	15.4 (n=499)	10	46	443	0.18
DZ and Sibling matched to MZ[‡]	18.2 (n=107)	4	12	91	0.25

*Six sibling pairs known to differ in CFTR genotype were excluded. Diabetes status of a pair was categorized as “Both” members having diabetes, “One” of two members having diabetes or “Neither” member having diabetes.

[†]Concordance for diabetes was defined as the number of pairs in which Both members had diabetes divided by the number of pairs in which One or Both had diabetes.

[‡]Same-sex DZ twin pairs combined with same-sex, age-corrected sibling pairs. Age correction was done by considering the diabetes status of the older member at the age of the younger member. From this

group were selected 136 pairs matched for age, sex, and pancreatic insufficiency to the 68 MZ pairs. Pairs did not differ in age ($p=0.4$) or any other matched variable.

§Pairs in which exactly one member had steroid treatment > 30 days (i.e., discordant for steroid treatment) were excluded.

||Pairs in which exactly one member had ABPA were excluded.

FIGURE 1

