Genetic Determinants and Epidemiology of Cystic Fibrosis-Related Diabetes - Results from a British Cohort of Children and Adults

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Objective: Longer survival of patients with cystic fibrosis has increased the occurrence of cystic fibrosis-related diabetes (CFRD). This study documents the incidence of CFRD and evaluates the association between mutations responsible for CF and incident CFRD, while identifying potential risk factors.

Research Design and Methods: Population-based longitudinal study of 50 CF speciality clinics in the United Kingdom. Subjects included 8,029 individuals aged 0 – 64 years enrolled on the UK CF Registry during 1996 – 2005. 5,196 with data and without diabetes were included in analyses of incidence, and 3,275 with complete data in analyses of risk factors. Diabetes mellitus was defined by physician diagnosis, oral glucose tolerance testing, or treatment with hypoglycemic drugs.

Results: 526 individuals developed CFRD over 15,010 person-years. The annual incidence was 3.5%. The incidence was higher in females and in patients with mutations in CF transmembrane conductance regulator (CFTR) gene in classes I and II. In a multivariate model of 377 cases of 3,275 patients, CFTR class (RR 1.70, 1.16 - 2.49, class I or II vs. others), increasing age, female sex, worse pulmonary function, liver dysfunction, pancreatic insufficiency, and corticosteroid use were independently associated with incident diabetes.

Conclusions: The incidence of CFRD is high in Britain. CFTR class I and II mutations increase the risk of diabetes independent of other risk factors including pancreatic exocrine dysfunction.
Considerable improvement in survival of patients with cystic fibrosis (CF) has led to the emergence of complications, one being cystic fibrosis-related diabetes (CFRD). CF, an autosomal recessive disease caused by the presence on each gene of at least one of over 1500 mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), is characterized by chronic pulmonary infections, pancreatic insufficiency, biliary cirrhosis, low body mass index (BMI), and, increasingly, CFRD. The prevalence of diabetes among European children and adults with CF now approximates 12%, rising to 30% in adults screened for diabetes. (1-3)

Unlike type 1 diabetes, patients with CFRD rarely develop ketoacidosis, but like type 2 diabetes, have elements of both decreased insulin secretion and sensitivity. Compared to other types of diabetes, the risk factors for CFRD are less well characterized; it is not entirely clear which factors differentiate individuals with CF who do, or do not, develop diabetes. Whereas genetic factors increase the prevalence, (4; 5) and likely the incidence, of CFRD, we do not know whether they do so independent of other identified risk factors.

Most observational studies of CFRD are cross-sectional, (3-7) and few prospective studies exist (2; 8). Risk factors identified include, among others, female sex, age, pancreatic insufficiency, poor pulmonary function, organ transplantation, elevated plasma fibrinogen and CFTR genotype. Cross-sectional designs cannot exclude the possibility that diabetes itself modifies some of these factors. The design would also obscure the true association between any risk factors associated with shortened survival and CFRD. (9) Likewise, no large longitudinal study has evaluated genetic factors associated with death, and, potentially, incident CFRD. (7)

To support research and care in CF, a number of national registries have been established including one in the United Kingdom in 1995 supported and coordinated by the Cystic Fibrosis Trust. Using data from the UK Cystic Fibrosis (UKCF) Registry, this study estimated incidence and identified risk factors for CFRD, with specific consideration for CFTR mutations, from a cohort of over 5,000 children and adults with CF.

METHODS
Subjects: This study identified 8,029 individuals aged 0 – 65 years entered on to the registry from 1996 to 2005. Data were routinely collected after patient consent in a standardized fashion from 50 British CF specialist centres. Of 8,029 patients, 6,678 had complete baseline data defined as registration followed within the same calendar year by a visit consisting of an annual review plus a clinic visit. Of these 6,678 patients, 761 (11.4%) with diabetes were excluded. Of the 5,917 individuals without diabetes, 721 lacked further follow-up leaving 5,196 with at least one annual follow-up visit. 3,275 patients (aged 2.0 – 55.3 years) had complete data for all covariates. Patients with complete data (compared to those without) did not differ by sex, but were older (median 14.1 years vs. 5.2 years p <0.0001), due mainly to the difficulty of acquiring pulmonary function testing in babies and young children.

Endpoint and Potential Risk Factors: Diabetes was defined as a physician diagnosis of diabetes, plasma glucose values consistent with diabetes
from oral glucose tolerance testing (OGTT), or treatment with insulin or oral hypoglycemic drugs.

Risk factors examined included age, sex, ethnicity, age of diagnosis of CF, respiratory infections, BMI, and pancreatic, hepatic or pulmonary dysfunction. They also included supplemental feeding, prior organ transplantation, corticosteroid use, and whether CF was detected via screening and CFTR genotype. Ethnicity was determined by the ethnicity of the parents. BMI (kg/m²) was calculated as weight divided by the square of the height. BMI z-scores were calculated using a UK reference population. (10) Pancreatic dysfunction was defined as use of pancreatic enzyme supplementation, and hepatic dysfunction as portal hypertension, abnormal liver function tests, or use of the bile acids (ursodeoxycholic acid or taurine). Pancreatic enzyme supplementation was expressed as total daily dose of the lipase component and as daily dose per kg of body weight. Supplemental feeding included oral supplements, and feeding via nasogastric, gastrostomy, or parenteral routes. An individual was considered infected with bacteria or fungi if cultured from sputum at baseline or if a physician had diagnosed chronic infection from at least two isolates in the year prior to baseline. Organisms included *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Burkholderia cepacia*, *Haemophilus influenzae*, *Methicillin-resistant staphylococcus aureus* (MRSA) and *Aspergillus fumigatus*. Allergic broncho pulmonary aspergillosis (ABPA) was diagnosed clinically. Pulmonary function was measured using forced expiratory volume at one second (FEV₁) and forced vital capacity (FVC) expressed as percentage predicted. (11)

Genotypes associated with CF were coded into five established classes reflecting CFTR function of defective production, processing, regulation, conductance and quantity of CFTR protein (12) as follows:

I: G542X, R553X, W1282X, R1162X, 621-1G→T, 1717-1G→A, 1078ΔT, 3659ΔC
II: ΔF508, ΔI507, N1303K, S549N
III: G551D, R560T
IV: R117H, R334W, G85E, R347P,
V: 3849+5G→A, A455E,
Unknown: 711+IG→T, 2184DA, 1898+IG→A

Patients with two alleles within the same class, or only one allele typed, were assigned that class. Heterozygotes for ΔF508 were assigned the class of the non-ΔF508 allele. (13)

**Statistics:** Incidence rates for CFRD were calculated as the number of new cases divided by follow-up time among the 5,196 patients with baseline and follow-up data, and stratified by 10-year age groups and sex. In this retrospective cohort, follow-up time lasted from registration to the first detection of diabetes or censoring, and was expressed in person-years.

Among the 3,275 individuals with complete data, we tested differences between patients who did and did not develop diabetes with chi-square tests for categorical variables, and t-tests or Kruskal-Wallis tests for normally or non-normally distributed continuous variables. Proportional hazards modeling identified potential risk factors. For categorical variables, we evaluated proportional hazards assumptions using Kaplan-Meyer survival curves. We chose variables for multivariate modeling if associated with CFRD in univariate
analyses at p ≤ 0.05. Age at baseline, BMI, BMI-z, and FEV₁ were coded as continuous. Binary variables included sex, ethnicity (at least one non-white parent vs. two white parents), hepatic dysfunction, supplemental feeding, respiratory infections, APBA, oral pancreatic enzyme supplementation and corticosteroid use (oral and/or inhaled) within the year prior to registration. Age of diagnosis of CF and dose of pancreatic enzyme supplementation were coded as binary variables around the medians. We coded CFTR genotype by class as described and as a binary variable (class I-II, vs. III -V). One-way interactions between genotype or sex and other variables were tested. We calculated hazard ratios (expressed as relative risks) with 95% confidence intervals (CI). We performed database manipulations using Caché (InterSystems, 2007), and statistical analyses using R (R Development Core Team, 2007).

RESULTS

The median age of the cohort was 12.0 years (interquartile range 6.0 – 19.8). 54% were male and 96% were white. The characteristics of the study population and of those who did or did not develop CFRD are shown (Table 1).

Incidence: Of 5,196 patients, 526 developed diabetes during 15,010 person years of follow-up (median 2.67 years, range 0.08 – 8.33). 40% (211) were diagnosed on the basis of insulin use, 22% (117) from OGTT results, 4% (20) on the basis of use of oral hypoglycemic drugs, and 1% (5) by physician diagnosis. The remainder met multiple criteria. The incidence of diabetes was 3.5% per year. During follow-up, 202 patients died without having been diagnosed with diabetes.

The incidence rose with age, was higher in females up to age 40, and declined after 40 for both men and women. Annual incidence rose from one to two percent in the first decade, to approximately six to seven percent in the fourth decade (Figure 1).

Risk Factors: Patients with class I mutations had the highest incidence of CFRD and class V mutations the lowest (Fig 2). The incidence was significantly higher in individuals with class I or II relative to III-V (p<0.01). In univariate analyses, patients with incident CFRD were less likely to have been screened for CF, and more likely to have infections, liver or pulmonary function abnormalities, and to have used corticosteroids or pancreatic enzyme replacement. The median FEV₁ in patients who developed CFRD was 57.8% compared to 75.3% in those who did not (p<0.0001). Adjusted for age and/or sex, FEV₁ remained strongly inversely associated with incident CFRD. BMI was higher, and BMI-z scores lower, in those who developed diabetes than in those who did not (Table 1).

In a multivariate model, increasing age, female sex, decreasing FEV₁, liver dysfunction, pancreatic enzyme replacement, corticosteroids use, and CFTR genetic class were independently associated with incident diabetes. CFTR class I-II relative to III-IV was associated with a relative risk of 1.70. Females were approximately 60% more likely to develop diabetes than males, and each year of age or percent decrease in predicted FEV₁ was associated with a two percent increase in risk (Table 2). Diagnosis of CF by screening, pulmonary infections, supplemental feeding, and BMI or BMI z-score were not associated with CFRD in multivariate modeling. There were no significant interactions.
Excluding 334 individuals not taking pancreatic enzymes, a multivariate model (362 cases among 2,931 patients) showed no association between the dosage of pancreatic enzyme replacement and CFRD.

**DISCUSSION**

This large, longitudinal, registry-based study documents a high incidence of CFRD among individuals with CF in Britain, and demonstrates that CFTR mutation class independently increases risk. The study confirms that females are at higher risk for CFRD, which is not accounted for by a higher prevalence of risk factors in females. The study confirms the independent associations for poor hepatic and pulmonary function, and for use of pancreatic enzymes or corticosteroids.

Few studies of CFRD incidence exist. The annual incidence of 3.5% we report is similar to the 3.8% reported from a Danish study of fewer than 200 patients. (2) Based on extremely small numbers (12 and 6 incident cases of CFRD) and derived from cumulative incidence values, Italian (8) and Greek (14) investigators show a CFRD annual incidence of approximately 4.0%. The high incidence of CFRD in the first decade in this study suggests that screening for diabetes in this age group, although not currently recommended in the UK, may be justified. Whether screen-detected individuals fare better is not determined.

CFTR protein regulates the function of chloride channels on the apical membrane of epithelial cells and helps regulate transepithelial transport of other ions and water. The grouping of mutations into functional categories are: (I) no synthesis of CFTR, (II) degradation of CFTR in the endoplasmic reticulum, (III) transport of CFTR to the cell membrane, but no appropriate response, (IV) diminished action of CFTR in the cell membrane and (V) normal, but inadequate amounts of, CFTR. (12) Many studies document associations between functional class and complications of CF, including pancreatic dysfunction (13; 15). This study shows an association between class and CFRD adjusted for pancreatic exocrine function as measured by pancreatic enzyme replacement. Investigators have previously searched for genes in CFRD, concentrating either on those associated with type 2 diabetes and inflammation or with CF. ΔF508 homozygosity was associated with CFRD in some, but not all, studies (3; 16-19). A prospective study comprising few patients with incident diabetes (n=12) (8) showed that patients homozygous for ΔF508 were more likely to develop diabetes. A large US registry-based cross-sectional study reported an association of ΔF508 homozygosity and CFRD, but presented only univariate results. (4) A European registry study documented a greater prevalence of diabetes in adults among class II (282/1276, 22.1%) mutations than in class IV mutations (1/65, 1.5%); however, no statistics testing were performed, nor an incidence rate calculated. (5) The present study included 377 CFRD cases, controlled for confounding factors, and, being longitudinal, accounted in part for competing risks. (13) It could not, however, determine, whether the risk factors caused diabetes.

Genotype may predispose to diabetes via pancreatic dysfunction or may play a more direct role. CFRD results more from decreased beta-cell function than decreased insulin sensitivity. (19) This supports a function
for CFTR in pancreatic islets where in rats investigators have identified CFTR mRNA. (20) In mice made diabetic by streptozotocin, those who were CFTR null (-/-) had higher blood glucose levels than other mice; the investigators concluded that islet dysfunction is inherent to the CFTR (-/-) state. (21) CFTR has also been identified in the human hypothalamus, (22) which may play a role in glucose regulation.

Whether pancreatic exocrine dysfunction mirrors pancreatic endocrine dysfunction is not clear. Investigators have found pancreatic exocrine dysfunction in both type 2 diabetes and type 1 diabetes. In CF, clinicians adjust doses of pancreatic enzyme in order to enable patients to eat a diet containing fat and diminish gastrointestinal symptoms. However, studies in CF have shown both under- and overtreatment with pancreatic enzymes relative to native pancreatic function. (23) The strong association between pancreatic enzyme use and incident diabetes in this study suggests that enzyme use is likely to be a good marker of pancreatic function, but may itself increase risk.

The association between female sex and CFRD is well described (4) and differs from type 1 or 2 diabetes. Earlier puberty with glucose intolerance has been proposed as an explanation, (24) and may in part account for our findings, since incidence in females rose between age five and ten (not shown). Yet, the female predominance persists beyond puberty. The absence of a female predominance over age 30 suggests that women susceptible to diabetes will have developed it, or may have died.

An association between poor pulmonary function and CFRD has been reported from cross-sectional studies. (4) (6) (5) and other studies have demonstrated accelerated decline in pulmonary function prior to diagnosis of CFRD. (2; 25) In CF, it is possible that pre-diabetic (or pre-diagnostic) states worsen pulmonary function. In the present study, poor pulmonary function preceded diabetes and was independent of age, sex, genotype, medical interventions and other CF-related complications. Individuals with diabetes (but without CF) have lung parenchymal histological changes including thickened basement membrane, fibrosis, and septal obliteration (26) suggesting a direct deleterious effect of hyperglycemia.

Other results are notable. Although CF is characterized by a low BMI, this did not itself increase the risk of diabetes. Corticosteroid use was independently associated with diabetes, but ABPA and organ transplantation, both indications for corticosteroids, were not, even in univariate analyses.

This study’s strength included was the use of data that were longitudinal, national, and systematically collected. With respect to possible biases related to incidence rates, the true incidence of CFRD in Britain may be higher than we report for three reasons. The calculation of time-to-diabetes overestimated true values, since patients developed diabetes prior to their clinic visits. Secondly, not all individuals were screened for diabetes. The UK CF Trust recommends annual OGTTs during periods of clinical stability, (17) and over 85% (20/23) of adult centers screen for diabetes. For pediatric centers, screening is recommended over age 12. Lastly, patients excluded from analysis of incidence were older (~ one year) and had worse pulmonary function (FEV₁ ~2% lower), and therefore were at higher risk for CFRD. While incidence rates derived from registries may be less
reliable than those from cohort studies, frequent follow-up of patients with CF in the UK (because of disease severity and free care) diminishes this possibility. Despite a longitudinal design, the relative risk we report for genotype may underestimate the true value if patients at high risk for diabetes died prior to registration, or were less likely to undergo genotyping. For each of genotype, corticosteroid use, pancreatic enzyme replacement, poor pulmonary function, and poor hepatic function, ascertainment bias is possible. If sicker patients had more frequent clinical visits, then their physicians may have been more likely to diagnose diabetes, and would mean that the relative risks we report overestimate true values.

In summary, this study documents the high incidence of diabetes mellitus in CF and that CFTR mutation class influences the risk of diabetes independent of other risk factors. It suggests that screening for diabetes may be merited across the age spectrum. By establishing age-specific incidence rates, this study provides useful information to health care providers, disease modellers and clinical trialists. By identifying risk factors, this study may help better understand the pathogenesis of CFRD.

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Table 1. Characteristics of Patients at Baseline stratified by Development of Diabetes. Absolute values (medians) for continuous variable with inter-quartile range and percentages for categorical variables. P-values represent difference between groups using Kruskal-Wallis for continuous variables and chi-square for categorical variables.

| Parameter                                      | All patients | Did not develop diabetes | Incident Diabetes | p-value |
|------------------------------------------------|--------------|---------------------------|-------------------|---------|
| Age (years)                                    | 14.1 (9.2 – 21.1) | 13.5 (8.9 – 20.5)          | 18.0 (12.4 – 25.4) | < 0.0001 |
| Sex (number (no.) female)                      | 1491 (45.5%) | 1288 (44.4%)               | 203 (53.8%)        | < 0.001 |
| Ethnicity (no. white)                          | 3166 (96.7%) | 2797 (96.5%)               | 369 (97.9%)        | n.s.    |
| BMI (kg/m²)                                    | 18.6 (16.4 – 21.3) | 18.5 (16.3 – 21.3)         | 19.2 (17.3 – 21.4) | < 0.001 |
| BMI z-score                                    | -0.15 (-0.86 – 0.56) | -0.10 (-0.81 – 0.59)     | -0.49 (-1.23 – 0.22) | < 0.0001 |
| FEV₁ (% predicted)                             | 72.9 (53.0 – 91.3) | 75.3 (55.3 – 92.7)         | 57.8 (36.9 – 74.5) | < 0.0001 |
| FVC (% predicted)                              | 82.6 (68.0 – 94.1) | 83.6 (69.6 – 95.3)         | 73.3 (57.1 – 86.9) | < 0.0001 |
| Any use of pancreatic enzyme (no.)             | 2,941 (89.8%) | 2,579 (89.0%)               | 362 (96.0%)        | < 0.001 |
| Lipase dose (units/kg of body weight/day)      | 6,829 (3,464 – 10,200) | 6,828 (3,448 – 10,150)    | 6,865 (3,602 – 10,560) | n.s.    |
| Supplemental feeding (no.)                     | 1,439 (43.9%) | 1,236 (42.7%)               | 203 (53.8%)        | < 0.0001 |
| Oral corticosteroids (no.)                     | 313 (9.6%) | 263 (9.1%)                   | 50 (13.3%)         | 0.012   |
| Any corticosteroids use (no.)                  | 1,761 (53.8%) | 1,526 (52.7%)               | 235 (62.3%)        | < 0.001 |
| Former organ transplantation (no.)             | 44 (1.3%) | 37 (1.3%)                    | 7 (1.9%)           | n.s.    |
| Abnormal liver function tests (no.)            | 406 (12.4%) | 342 (11.8%)                  | 64 (16.9%)         | <0.01   |
| Use of ursodeoxycholic acid or taurine (no.)   | 534 (16.3%) | 455 (15.7%)                  | 79 (21.0%)         | 0.012   |
| Any hepatic dysfunction (no.)                  | 719 (28.1%) | 610 (21.0%)                  | 109 (28.9%)        | < 0.001 |
| Pseudomonas aeruginosa (no.)                   | 1925 (58.8%) | 1651 (57.0%)                 | 274 (72.7%)        | < 0.0001 |
| Burkholderia cepacia (no.)                     | 151 (4.6%) | 129 (4.5%)                   | 22 (5.8%)          | n.s.    |
| Staphylococcus aureus (no.)                    | 1379 (42.1%) | 1223 (42.2%)                 | 156 (41.4%)        | n.s.    |
| Haemophilus influenzae (no.)                   | 668 (20.4%) | 605 (20.9%)                  | 63 (16.7%)         | n.s.    |
| Aspergillus fumigatus (no.)                    | 341 (10.2%) | 301 (10.2%)                  | 40 (10.6%)         | n.s.    |
| MRSA (no.)                                     | 46 (1.4%) | 40 (1.4%)                    | 6 (1.6%)           | n.s.    |
| ABPA (no.)                                     | 197 (6.0%) | 178 (6.1%)                   | 19 (5.0%)          | n.s.    |
| CFTR mutation class (no.)                      | 266 (8.1%) | 227 (7.8%)                   | 39 (10.3%)         | < 0.01  |
| I                                              | 2608 (79.6%) | 2299 (79.3%)                | 309 (82.0%)        | n.s.    |
| II                                             | 256 (78.2%) | 231 (8.0%)                   | 25 (6.6%)          | n.s.    |
| IV                                             | 125 (3.8%) | 121 (4.2%)                   | 4 (1.1%)           | n.s.    |
| V                                              | 20 (0.6%) | 20 (0.7%)                    | 0 (0%)             | n.s.    |
| unknown                                       | 0 (0%)    | 0 (0%)                       | 0 (0%)             | n.s.    |
| CFTR mutation class I or II (no.)              | 2874 (87.7%) | 2526 (87.2%)                | 348 (92.3%)        | <0.01   |
| Age at diagnosis of CF in years                | 0.4 (0.08 – 2.5) | 0.4 (0.08 – 2.5)          | 0.5 (0.08 – 2.0)   | n.s.    |
| CF detected by screening (no.)                 | 377 (11.5%) | 349 (12.0%)                  | 28 (7.4%)          | 0.011   |
Table 2 Multivariate risk factor model and models comprised of one independent variable for CFRD. 377 cases incident diabetes of n = 3,275

| Risk Factor                  | Reference            | RR   | 95% CI     | RR   | 95% CI     |
|------------------------------|----------------------|------|------------|------|------------|
|                              | Multivariate model   |      |            |      |            |
|                              | Univariate models    |      |            |      |            |
| Age                          | each year older      | 1.03 | 1.01-1.04  | 1.05 | 1.04 – 1.06|
| Female sex                   | male                 | 1.62 | 1.32 – 1.99| 1.37 | 1.15 – 1.63|
| FEV₁                         | each % worse         | 1.018| 1.013 - 1.023| 1.022| 1.018-1.026|
| Liver dysfunction            | vs. absent           | 1.41 | 1.12 – 1.77| 1.66 | 1.36-2.03  |
| Any pancreatic enzyme use    | vs. none             | 2.97 | 1.73 – 5.09| 1.34 | 1.1-1.63   |
| Any corticosteroid use       | vs none              | 1.24 | 1.002 – 1.53| 1.65 | 1.39-1.97  |
| CFTR Class                   | I/II vs III/IV/V     | 1.70 | 1.16 – 2.49| 4.58 | 1.81-13.0  |
Legends for Figures

Fig. 1 Incidence of cystic fibrosis related diabetes per 100 person years by age and sex. Vertical bars represent 95% confidence limits. Males represented in black, and females in grey.

Fig. 2 Incidence of cystic fibrosis related diabetes per 100 person years by class of CFTR mutation. Vertical bars represent 95% confidence limits.
