Cystic fibrosis–related diabetes onset can be predicted using biomarkers measured at birth

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PURPOSE: Cystic fibrosis (CF), caused by pathogenic variants in the CF transmembrane conductance regulator (CFTR), affects multiple organs including the exocrine pancreas, which is a causal contributor to cystic fibrosis–related diabetes (CFRD). Untreated CFRD causes increased CF-related mortality whereas early detection can improve outcomes.

METHODS: Using genetic and easily accessible clinical measures available at birth, we constructed a CFRD prediction model using the Canadian CF Gene Modifier Study (CGS; n = 1,958) and validated it in the French CF Gene Modifier Study (FGMS; n = 1,003). We investigated genetic variants shown to associate with CF disease severity across multiple organs in genome-wide association studies.

RESULTS: The strongest predictors included sex, CFTR severity score, and several genetic variants including one annotated to PRSS1, which encodes cationic trypsinogen. The final model defined in the CGS shows excellent agreement when validated on the FGMS, and the risk classifier shows slightly better performance at predicting CFRD risk later in life in both studies.

CONCLUSION: We demonstrated clinical utility by comparing CFRD prevalence rates between the top 10% of individuals with the highest risk and the bottom 10% with the lowest risk. A web-based application was developed to provide practitioners with patient-specific CFRD risk to guide CFRD monitoring and treatment.

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INTRODUCTION

Genome-wide association studies (GWAS) have been successful at identifying genetic contributors to disease,1 however, clinical utility of GWAS findings has been slow to follow. One explanation is that the genetic architecture of complex phenotypes is multifaceted and individual GWAS findings have small effect sizes that limit their potential alone as predictors of disease.2 Although GWAS has provided us with important mechanistic insight into disease, further defining genetic markers for risk prediction could have significant impact on personalized medicine. Here, we investigate genomic-based risk prediction for cystic fibrosis–related diabetes (CFRD).

Cystic fibrosis (CF) is a life-limiting genetic disease caused by loss-of-function pathogenic variants in the cystic fibrosis transmembrane conductance regulator (CFTR) and affects multiple organs including the exocrine pancreas. Pancreatic damage and the resulting exocrine pancreatic insufficiency (PI) contribute to CFRD,3 which is seen in 19% of adolescents and 40–50% of CF individuals by age 40.4 CFRD is associated with increased morbidity due to worsening lung and nutritional status, which often precedes CFRD diagnosis, and increased mortality if CFRD remains untreated.4 Early identification could improve clinical outcomes and reduce mortality.5 Current guidelines recommend annual CFRD screening with 2-hour oral glucose tolerance testing (OGTT) after 10 years of age; however, there is poor adherence...
with screening rates reported below 50%.6 Identifying individuals at greatest risk of developing CFRD as early as possible could improve adherence.

CFRD occurs predominantly in individuals with severe CFTR pathogenic variants that result in PI.7 Thus, currently the best predictor of CFRD risk is whether an individual has CFTR pathogenic variants associated with PI (85% of the CF population8); however, we expect variation in risk even within individuals that are PI. In addition to the CFTR contribution, GWAS has identified genetic modifiers of CFRD at SLC26A9 and several established type 2 diabetes susceptibility loci.9,10 Consistent with PI CFTR variants’ elevating risk for CFRD, recent studies have suggested a major cause of CFRD to be prenatal and early postnatal damage to the exocrine pancreas.3 The degree of pancreatic damage and reduction in acinar tissue are reflected by circulating immunoreactive trypsinogen (IRT), which is partially encoded by serine protease 1 (PRSS1). Newborn-screened (NBS) IRT and its longitudinal measures in the first 2 years of life have been shown to associate with CFRD risk in two independent samples.3 However, routine longitudinal measurement of IRT is not standard of care for young CF individuals and is unavailable for older CF individuals who were diagnosed later in life but are at greatest CFRD risk today. Therefore, this study aims to identify biomarkers that can predict CFRD onset using genetic and easily accessible clinical measures early in life. With the Canadian CF Gene Modifier study (CGS), we developed a prediction model to identify individuals at highest risk of CFRD at different ages and validated our prediction in an independent CF cohort from France.

MATERIALS AND METHODS

Demographics, genotyping, and phenotyping

Two independent population-based cohorts were included in this study: the CGS (n = 1,958) and the French CF Gene Modifier Study (FGMS, n = 1,003). CGS was used to develop the predictive model while FGMS was used to validate the predictions. Ninety-seven percent of the CGS participants included in this study were diagnosed with CFRD in the CGS. Specified as CFTR inheritance or having SMN1 variants associated with type 2 diabetes in the general population,12 we did not include individuals without a CFRD diagnosis or have time varying BMI to be a strong predictor of future CFRD risk and we removed it from the analyses.

In CF, the standard of care is to employ annual OGTT testing to conclude the presence of CFRD, but there is poor adherence to this time-consuming test that requires an overnight fast.13 In the CGS, CFRD status was determined using a combination of chart review and the Canadian CF patient registry.2 Patients diagnosed with CFRD had a physician’s

| Variable                          | Canadian GMS (n = 1,958) | French GMS (n = 1,003) |
|-----------------------------------|--------------------------|------------------------|
| CFRD (cases)                      | 619 (31.6%)              | 374 (37.3%)            |
| Sex (females)                     | 926 (47.3%)              | 480 (47.9%)            |
| Meconium ileus                    | 334 (17.1%)              | 141 (14.1%)            |
| Newborn screened                  | 58 (3.0%)                | 415 (42.5%)            |
| CFTR variant score                |                          |                        |
| 5                                 | 51 (2.6%)                | 14 (1.4%)              |
| 4                                 | 389 (19.9%)              | 201 (20.0%)            |
| 3                                 | 1185 (60.5%)             | 667 (66.5%)            |
| 2                                 | 170 (8.7%)               | 68 (6.8%)              |
| 1                                 | 163 (8.3%)               | 53 (5.3%)              |
| Age cohort (year of birth)        |                          |                        |
| 1970s                             | 336 (17.2%)              | 128 (12.8%)            |
| 1980s                             | 634 (32.4%)              | 317 (31.6%)            |
| 1990s                             | 737 (37.6%)              | 392 (39.1%)            |
| After 2000                        | 251 (12.8%)              | 166 (16.6%)            |

Individuals enrolled in the FGMS are less likely to carry a mild CFTR pathogenic variant compared with participants in the CGS. CFRD cystic fibrosis-related diabetes, GMS Gene Modifier Study.

November Seven French GMS individuals were missing information for newborn screening. A higher proportion of French individuals were newborn screened since nationwide newborn screening was implemented in France in 200237, earlier than that in all Canadian provinces and territories.

For the predictive model we evaluated a set of 3,984 single-nucleotide polymorphisms (SNPs) that were annotated to genes previously identified as CF modifiers. These included genes that code for proteins residing at the apical plasma membrane alongside CFTR17,18; variants identified as genetic modifiers of CFRD9 or SNPs associated with other common CF comorbidities including MI11 and lung function decline.19

To address the potential for population stratification in the CGS training data, we used KING20 to perform principal component analysis (PCA). SNPs with minor allele frequency greater than 0.05 and with low pairwise linkage disequilibrium (r² < 0.2) were included. The Tracy-Widom test determined that ten principal components (PCs) were statistically significant (p < 0.01) in the CGS and were incorporated as predictors in feature selection and model fitting (Appendix B). The lack of differences in model performance with and without adjustment for the PCs (Appendix C)
suggested confounding due to population structure in the CGS. Moreover, both studies are ethnically homogeneous (>94% Europeans) with non-Europeans defined as >3 SD from the center of the 1000 Genomes European cluster (Appendix D).

The variables included in model training consisted of the 3,984 preselected SNPs, MI, sex, CFTR severity score, and the first ten PCs.

Developing risk scores for CFRD

With the goal of predicting CFRD, all 1,958 individuals in the CGS were included to construct a prediction model that was then validated on the independent FGMS cohort (n = 1,003). To compare model performance across the two independent studies, we performed internal cross-validation within the CGS to reduce overfitting. Since using a single pair of training and validation sets can produce overly optimistic results, we randomly partitioned 1,958 participants into a training (n = 1,300) and a validation set (n = 658) and repeated this partition 500 times. Model fitting was based solely on the training sets while the validation sets were used to assess model performance. We also calculated 95% confidence intervals (CI) for predictive accuracy at specified ages.

CFRD risk was modeled in a three-stage approach: (1) hierarchical clustering to remove highly correlated SNPs; (2) stability selection21 and component-wise gradient boosting22 to rank variable importance by their cutoffs (0.05, 0.01, 0.005, 0.001, 0.0005, 0.0001; Appendix F). The PRS MI and cohort to ensure a fair comparison.

A random effects model was used to re-estimate overpenalized effect sizes23 (Appendix J). We ranked variable importance by stability selection using all genes that contribute to exocrine pancreatic disease severity are strongly associated with CFRD risk; and (3) Cox proportional hazards (Cox PH) selected frequencies, with a 50% cutoff used to select predictors most strongly associated with CFRD risk, with genotype information beyond the T allele at rs7903146 also show greater susceptibility to CFRD. The effect size of the variant in the FGMS. Wider CIs can also be observed for other variants in the FGMS. Consequently, the ability of our model to stratify CFRD risk based on the severity score is by far the best predictor of CFRD risk after adjusting for CFRD risk score (Supplementary Fig. 4, red curve) are shown in Fig. 1b as greater than 96% of the SNPs evaluated were figure as greater than 96% of the SNPs evaluated were

RESULTS

We calculated the CFRD-free probabilities and their 95% CIs at different ages for Canadians (CGS) with different CFRD severity scores (Appendix E). CFRD-free probabilities for individuals with the least severe CFRD score (Supplementary Fig. 4, red curve) are higher than the other groups across all ages. In contrast, CFRD-free probabilities for individuals with other CFRD scores either overlap extensively (scores 2 to 4) or cannot be reliably estimated due to the smaller sample size (score 5). To avoid excess uncertainty in the fitted model, we dichotomized CFRD scores into a high (scores 2 to 5) and a low (score 1) group for all subsequent analyses rather than using an ordinal scale; this choice had little impact on the final model performance (Appendix G).

We ranked variable importance by stability selection using all individuals in the CGS (Fig. 1a). Eight variables exceed the 50% threshold (red, Fig. 1a). The CFRD severity score is by far the strongest predictor (hazard ratio [HR] 95% CI: [2.01, 4.54]), selected in 100% of the stability selection subsets. Sex and cohort effect are the second and third most important variables for predicting CFRD risk, both chosen in 92% of the subsets. SNPs annotated to genes that contribute to exocrine pancreatic disease severity are also ranked highly as predictors including rs4077468 annotated to the previously identified MI and CFRD modifier SLC26A9 (HR 95% CI: [1.07,1.34]) and rs1964986 annotated to PRSS1 (HR 95% CI: [1.09,1.38]). PRSS1 encodes cationic trypsinogen and has not been reported to associate with CFRD, although it has been previously associated with MI in CF.11

In addition to the predictors that exceed the predefined threshold (Fig. 1a, red), we further included known CFRD risk factors or confounders to construct the final prediction model. These include the ten PCs to adjust for population structure; rs7903146 (TCF7L2; Fig. 1a, blue), an established type 2 diabetes gene27 that was ranked highly among the predictors even if it did not exceed the 50% threshold; and another highly ranked predictor, MI (Fig. 1a, rank 14, blue). MI is also correlated with exocrine pancreatic disease severity25,29, and was previously shown to be a marker of the known but not widely measured CFRD risk factor, NBS IRT.11 Although MI is associated with exocrine pancreatic disease severity, it remains associated with increased CFRD risk after adjusting for CFTR severity score in our model. Both MI and rs7903146 surpassed the majority of the SNPs not shown in the figure as greater than 96% of the SNPs evaluated were selected in less than 10% of the iterations.

Table 2 lists the HRs and the corresponding 95% CIs fitted in a multivariate Cox PH model after adjusting for cohort effects and the 10 PCs in the CGS. The risk allele or risk group is noted in parentheses. As expected, CF individuals carrying more severe pathogenic variants (higher CFRD scores) have much higher risk of CFRD. Females and individuals born with MI also exhibit higher CFRD risk. For the SLC26A9 SNP rs4077468, the A allele is associated with increased CFRD risk while CF individuals carrying the T allele at rs7903146 also show greater susceptibility to CFRD. The results indicate both genetic and clinical characteristics contribute to CFRD risk, with genotype information beyond CFTR improving the model’s explained variation in CFRD risk from 12% to 18% in the CGS.

Fig. 1b shows the time-dependent accuracy measure, AUC(t), for CGS and FGMS. The age-dependent model defined in the CGS shows excellent agreement when validated in the FGMS, demonstrating that our approach has selected stable predictors generalizable to other populations. The risk classifier also shows slightly better performance at predicting CFRD risk later in life (e.g., AUC = 0.71, age = 28 in FGMS) in both study cohorts. Of note, our model outperforms univariate PRS regardless of the chosen p value cutoff (Appendix F).

To further investigate model performance between CGS and FGMS, we plotted univariate log HR and the 95% CI for each selected predictor (Fig. 1c). Increase in CFRD risk for females and those with at least one copy of the type 2 diabetes risk allele (rs7903146[T]) show good agreement in both studies. Those with at least one copy of the PRSS1 (rs1964986[C]) and those with at least one copy of the SLC26A9 risk variant (rs4077468[A]) also show similar increases in CFRD risk in both independent data sets. However, several predictors including MI, the variants rs12318809 (SLC5A8), rs7822917 (NRG1), and rs959173 (CAV1) have much weaker effects in the FGMS. The effect size of the CFRD score is comparable in the FGMS and CGS, albeit with a wider CI for the FGMS since relatively fewer individuals carry mild CFTR pathogenic variants in the FGMS. Wider CIs can also be observed for other predictors due to a smaller sample size in FGMS. Consequently, the ability of our model to stratify CFRD risk based on the CFRD score may be underutilized in the FGMS and leads to underestimated performance at younger ages. Winner’s curse, in which the associations of selected predictors in the training data set are more likely to be overestimated, might also be a contributing factor.28

Since AUC(t) only measures a model’s ability to rank individuals based on their estimated risk, we further evaluated a more clinically relevant metric by comparing CFRD prevalence rates between individuals with the highest and lowest 10% risk.
DISCUSSION

We developed a model to estimate an individual’s CFRD risk using genetic and clinical measures available at birth. The final model
can differentiate individuals with varying CFRD risk with reasonable accuracy across different ages. The selected variables that are among the strongest predictors of CFRD risk—CFTR severity score, MI, and the genetic variants annotated to PRSS1 and SLC26A9—suggest that measures of exocrine pancreatic disease severity are major predictors of CFRD. These results are supported by findings from earlier studies that showed increased risk in those born with MI	extsuperscript{9} and that SNPs annotated to SLC26A9 are associated with CFRD	extsuperscript{9} through their impact on exocrine pancreatic damage.\textsuperscript{3,11}

The SLC26A9 variant (rs4077468) and MI were shown to associate with CFRD in a previous study using partially overlapping individuals from the CGS.\textsuperscript{9} However, the results were confirmed in our study using 555 (28%) new participants from the CGS and an independent French population cohort (FGMS) not included in the initial study.\textsuperscript{7,29} Investigating other factors independent of those associated with exocrine pancreatic damage, we found that females exhibit higher CFRD risk, consistent with previous findings;\textsuperscript{7,29} and the type 2 diabetes gene, TCF7L2, also ranks highly among the predictors.

Our application (https://predictcfrd.research.sickkids.ca/) can assist clinicians in determining an individual’s CFRD risk across the age spectrum from measures obtained one time as early as birth. The Cystic Fibrosis Foundation recommends universal annual screening for CFRD. Findings here should not impact the recommended annual screening, even for those predicted to have the lowest risk, as less frequent monitoring would likely have a negative impact, regardless of risk category. Poor adherence to annual screening has, however, hindered its efficacy. Providing a percentile of an individual’s risk estimate and the CFRD prevalence rates across ages would highlight individuals at greater risk earlier in their disease course and could motivate improved adherence to...
regular OGTT measurements, or perhaps greater frequency, for the high-risk subgroup at the discretion of their care provider.

We compared CFRD prevalence between individuals with the highest and lowest 10% risk since those at the tails of the risk distribution are most affected by clinical decision making.\(^3\)\(^2\) The model is capable of identifying individuals most susceptible to CFRD at different ages while maintaining a reliable estimation for those at low risk. In addition to age-distributed CFRD prevalence rates for each CF individual, age-dependent PPVs and NPVs using different thresholds for the CFRD high-risk category (Appendix H) serve to showcase the efficacy of the model and provide additional information to facilitate clinical decision making. Moreover, the results also demonstrate the benefit of genotyping modifiers in addition to the CFTR common causal variants in newborn screening programs, as incorporating modifier genotype information in addition to CFTR and clinical measurements (e.g., sex, MI, cohort) significantly increased the explained variation in CFRD risk (12% to 18%) in the CGS.

Despite taking extra precautions to avoid overfitting in our training data, winner’s curse might still contribute to overestimated effect sizes and lead to predictors being less robust in CFRD risk (12% to 18%) in the CGS. Highly effective upon the assumption that the CFRD diagnosis guidelines and emphasize early detection.

Moreover, the results also demonstrate the benefit of genotyping modifiers in addition to the CFTR common causal variants in newborn screening programs, as incorporating modifier genotype information in addition to CFTR and clinical measurements (e.g., sex, MI, cohort) significantly increased the explained variation in CFRD risk (12% to 18%) in the CGS.

In both the CGS and FGMS, the CFRD diagnosis data came from individual physicians. As most diagnoses are supported with OGTT, we do not expect significant impact from adopting a 7% cutoff for HbA1c compared with the general guideline of 6.5%.\(^4\) However, it is plausible that the use of a higher HbA1c cutoff in this study resulted in underdiagnosis in our analyzed cohorts. Moreover, although CFRD presents differently than T1DM, and T1DM and other forms such as maturity onset diabetes of the young (MODY) are rare in CF, it is possible that a small number of individuals may have been misrepresented as having CFRD.

We note a few limitations of this study, especially for the model’s use in clinical settings. The tool is designed to serve as an additional piece of information to enhance clinical care for CFRD and requires discretion by the clinical care provider to dichotomize CF individuals into high and low-risk groups based on the reported age-distributed prevalence. The CF gene modifiers are not routinely genotyped on CFTR diagnostic panels, and this change is needed to enable clinical use. The proposed model is constructed from measures obtained one time, as early as birth, and does not update risk predictions based on a patient’s current age or other longitudinal factors. Although a conditional risk model would be of interest, given the limited sample size and the corresponding stability of the model, we chose to focus on leveraging genetic and clinical measurements available at birth to emphasize early detection.

Although the model shows clinically relevant performance in stratifying CFRD risk among individuals in the Canadian and French studies, its clinical utility for future CF individuals relies upon the assumption that the CFRD diagnosis guidelines and prevalence remain static. Highly effective CFTR modulators could potentially affect the natural history of CFRD and reduce its prevalence in the modulator-treated population,\(^3\)\(^2\) although the impact of current therapies on pancreatic morbidity in CF remains unknown.\(^1\) Trikafta\(^\text{TM}\) has been approved for 90% of CF individuals, yet variability in its effectiveness has been reported.\(^3\)\(^4\) Moreover, it remains unavailable in many countries including Canada. Clinical utility in patients on highly effective CFTR modulators will need to be reinvestigated in future work.

Conclusion

CFRD is associated with poor prognosis in individuals with CF while early diagnosis and aggressive treatment contribute to improvements in survival.\(^4\) Thus, annual CFRD screening from 10 years of age is recommended.\(^3\)\(^5\) Despite these recommendations, compliance with testing is low.\(^3\)\(^6\) We have developed a model that estimates an individual’s CFRD risk at different ages over the course of their disease. The risk estimates can be used by clinical care providers to improve adherence to recommended annual screening or to trigger increased testing frequency. The hope is that improved adherence or more frequent testing will lead to earlier diagnosis and contribute to further gains in median survival that the CF population have been realizing over the last few decades.

DATA AVAILABILITY

Genotype data are available by application to the CF Canada National Data Registry for access to confidential clinical data for the purpose of CF research.

CODE AVAILABILITY

Code is available from the authors upon request.

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