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A Novel Lung Disease Phenotype Adjusted for Mortality Attrition for Cystic Fibrosis Genetic Modifier Studies

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SUMMARY

Genetic studies of lung disease in Cystic Fibrosis are hampered by the lack of a severity measure that accounts for chronic disease progression and mortality attrition. Further, combining analyses across studies requires common phenotypes that are robust to study design and patient ascertainment.

Using data from the North American Cystic Fibrosis Modifier Consortium (Canadian Consortium for CF Genetic Studies, Johns Hopkins University CF Twin and Sibling Study, and University of North Carolina/Case Western Reserve University Gene Modifier Study), the authors calculated age-specific CF percentile values of FEV1 which were adjusted for CF age-specific mortality data.

The phenotype was computed for 2061 patients representing the Canadian CF population, 1137 extreme phenotype patients in the UNC/Case Western study, and 1323 patients from multiple CF sib families in the CF Twin and Sibling Study. Despite differences in ascertainment and median age, our phenotype score was distributed in all three samples in a manner consistent with ascertainment differences, reflecting the lung disease severity of each individual in the underlying population. The new phenotype score was highly correlated with the previously recommended complex phenotype, but the new phenotype is more robust for shorter follow-up and for extreme ages.

A disease progression and mortality adjusted phenotype reduces the need for stratification or additional covariates, increasing statistical power and avoiding possible distortions. This approach will facilitate large scale genetic and environmental epidemiological studies which will provide targeted therapeutic pathways for the clinical benefit of patients with CF.

Keywords

Forced Expiratory Volume; Age Effects; Severity of Illness Index

INTRODUCTION

Although cystic fibrosis (CF) is a classic Mendelian disease, there is great variability in the chronic progressive lung disease which is the principal cause of early mortality. Variation in lung function, even among patients with the same CFTR mutations, is largely heritable, and modifier genes are known to influence pulmonary disease severity in CF¹⁻⁴. Large scale studies of the role of genetic variation in the severity of CF will potentially lead to novel therapeutic targets. However, for powerful analysis across the entire genome a phenotypic measure is required that will accurately represent individual disease severity for all ages, and across different populations and study designs. The pulmonary disease phenotype is usually based on forced expiratory volume in one second (FEV1), the most clinically useful measure of lung function and a known predictor of survival⁵⁻⁷. FEV1 is the volume of air (in liters) that can be exhaled in one second (after maximal inhalation)⁸ and is generally reported as a percent predicted (FEV1pp) based on age, sex, and height⁹⁻¹³. To address the concern that standard norms may be invalid in CF patients because of stunting and/or delayed puberty, Kulich et al. designed CF specific percentiles (CF FEV1%ile) to measure lung function relative to other CF patients of the same age, sex, and height¹⁴. Neither FEV1pp nor CF FEV1%ile account for mortality attrition; patients are compared only to those still alive, excluding patients from the same birth cohort who have died (usually because of more severe lung disease).

To derive a measure that accounted for mortality, Schluchter and colleagues developed a model combining mixed model regression and survival analysis, in which estimates of slope and intercept for an individual patient's rate of decline in FEV1pp could be used to predict

the age at death for that patient⁵. In an application of this complex model, Schluchter et al. concluded that estimated FEV1pp at age 20 based on a mixed model approach, wherein longitudinal FEV1pp values of many patients are analyzed together in a single regression model, was the most suitable of several phenotypes considered for modifier studies, equivalent to estimated percentile of predicted age of death¹⁵. Although this approach accounts for survival and has been useful, it has limitations for general application. These estimates are critically related to the number of measures of lung function, the number of years over which they were collected, the make-up of the study population, and the changing patterns of CF survival and lung function in recent decades.

In this paper we describe a novel approach to the development of a robust pulmonary disease phenotype that allows for both the effects of chronic disease progression and mortality attrition, and allows the use of less extensive longitudinal data.

METHODS

Patient study samples

We apply our approach to the North American CF Modifier Consortium which consists of three study groups whose ascertainment schemes differed. The Canadian Consortium for CF Genetic Studies (CGS) was designed to recruit a representative sample of the Canadian CF population to identify modifier genes. This sample of 2473 patients was collected from 37 Canadian CF centers between 2000 and 2004¹⁶. Children under the age of 2 years were excluded from the study to avoid enrolling newly diagnosed infants in a period of stress and variable compromise. Longitudinal data from each clinic visit in the three years preceding entry into the study included FEV1, age, height and weight.

The Johns Hopkins University CF Twin and Sibling Study (TSS) was family-based and included 1323 patients from 693 families who were recruited from 71 CF centers in the US and one in Australia⁴. Individuals with CF who had one or more living siblings with CF were eligible for inclusion. Patient samples were collected over a period of several years, most in 2002-2007. Although lifetime clinical information was collected, only the three years preceding study entry were analyzed herein.

The third sample, from the University of North Carolina at Chapel Hill/Case Western Reserve University Gene Modifier Study (GMS)², was an extremes of phenotype design. The sample included 1137 patients who were homozygous for F508del from 44 US CF centers who were enrolled between 2000 and 2006. Using data from the US CF Foundation Patient Registry to define quartiles of FEV1pp using the Knudson equations¹⁷, patients were recruited from one of three groups – severe (lowest quartile for age; 8-25 years); younger mild (highest quartile for age; 15-28 years); and older mild (highest quartile for ages 29-33 years plus all those at least 34 years, considered mild by virtue of survival). For each patient, data for the previous five years were collected, but only three years were used in the present analysis.

Entry criteria for subjects in all three studies included verification of CF diagnostic information, including elevated sweat tests and CFTR mutation analysis, according to standard guidelines¹⁸.

Phenotype derivation

Age-specific percentile scores—For the population-based CGS sample, mean age and mean FEV1pp were calculated using data from the three years prior to study enrollment. Pediatric (Wang et al.¹³) and adult (Hankinson et al⁹) reference equations were used as recommended by the US CF consensus group¹⁹ to calculate FEVpp at each visit. Residual

values from a non-linear regression of mean FEV1_{pp} vs. mean age (see Figure 1) were converted to rank percentile values to characterize the lung disease severity of each individual in the surviving population.

Because the TSS and GMS samples were not population based, we adapted the method described above, to account for variable designs and ascertainment schemes. We calculated the CF FEV1%ile (Kulich et al.) at each patient visit, and used the mean CF FEV%ile for each individual to rank him/her among CF survivors.

Computation of cohort survival estimates—The Canadian CF Patient Data Registry (CPDR) has collected prospective data on all Canadian CF patients since 1973²⁰. Patients included in the CPDR come from a network of audited CF care centers where the criteria for diagnosis of CF include elevated sweat chloride tests and/or identified CFTR mutations, and documentation of classic CF symptoms. Virtually all CF patients in Canada are captured in the CPDR, and the CGS sample is a representative subset. In order to compute birth cohort survival curves, de-identified birth and death dates, as well as date of last reported visit were requested on all patients in the 2002 CPDR, following ethics review. For patients born in 1973 or later, survival curves based on two year birth cohorts from the CPDR were created with a censoring date of 31 December 2002, the approximate midpoint of CGS data collection. Cohort cumulative survival probabilities on this date were used to create the survival estimates. For patients born before 1973, survival estimates were created by dividing the number of CF patients from each birth cohort alive on 31 December 2002 by the expected number of CF births (Canadian births x 1/2500). Local area regression was used to compute smoothed estimates of survival across the birth cohorts.

Adjustment of FEV1 percentiles for mortality—An individual's FEV1 percentile as computed above gave an estimate of the proportion of surviving patients of the same age, ie from the same birth cohort, whose lung function was below that individual's. Multiplying this number by the estimated survival percentile for that age provided the estimated proportion of patients from the entire original birth cohort who were surviving with FEV1 values below that of the individual. Adding this value to the proportion of patients from the original birth cohort who did not survive (1 minus the estimated survival percentile), gave an estimate of the proportion of patients from the individual's original birth cohort who had FEV1 values below that of the individual or who had died, presumably with more severe lung disease. Transformation of this mortality adjusted cohort percentile to a standard normal score yielded the Normal Residual Mortality Adjusted (NoRMA) lung disease phenotype. Application of the survival adjustment to mean CF FEV1%ile and transformation to a normal score produced the Kulich NoRMA (KNoRMA) phenotype. This general framework (Table 1) for FEV1 could be applied to any quantitative measure that varies with age and whose distribution suffers from mortality attrition.

Statistical methods

Local area regression (PROC LOESS in SAS version 9.1) was used to define age-specific non-linear regression estimates of FEV1_{pp} for NoRMA in the CGS sample, and to create the final smoothed age-specific survival estimates. Local area regression computes least squares regression of a dependent variable (here FEV_{pp}) on an independent variables (here age) in successive small neighbourhoods of the dependent variable and then splices these small linear regression lines together to produce a curve that is not constrained to any particular shape. FEV1_{pp} residuals from the LOESS curve were normally distributed at all ages and showed no age-specific patterns (data not shown), justifying the conversion of the mortality-adjusted percentile values to normal deviates using SAS PROC PROBIT. SAS PROC LIFETEST was used to compute birth cohort survival curves from the CPDR data. The three

years of data that were used to create NoRMA and KNoRMA were also used in a mixed model analysis (PROC MIXED) to compute two phenotypes proposed by Schluchter: the predicted value of FEV1pp at age 20 (FEVest20) and the predicted age at death percentile (AgeDied%ile)^{5, 15} based on patient specific slope and intercept. The MIXED procedure is a combination regression model allowing repeated measures for individuals, providing fixed effect coefficients for the overall effects of intercept and slope as well as random effect coefficients to calculate subject specific values for intercept and slope. Correlations between the phenotypes were investigated using PROC CORR.

Estimate of heritability for KNoRMA

Heritability was estimated using the TSS sample, which included twins as well as siblings. Heritability was calculated from 67 pairs of monozygous twins and 138 pairs of same-sex dizygous twins and non-twin siblings born within 3 years of each other. The pairwise correlation coefficient was calculated for each group, with assignment of twins or siblings as “A” or “B” permuted 10⁶ times. The average of the 10⁶ estimates for monozygous twins and for same sex dizygous twins or sibs was a robust estimate of the true correlation in each group, avoiding any bias that may have been introduced by arbitrary or systematic assignments to A and B status. Heritability was estimated to be twice the difference in the group mean coefficients⁴.

RESULTS

Population based CGS patient sample

Representativeness of the CGS sample was assessed by comparison to the 2002 CPDR Report (Table 2).

Reproducible and reliable FEV1 measurements are typically only obtained in patients six years and older. Thus, development of the NoRMA phenotype was based on 2061 patients (1104 males; 957 females), six years of age and older, with 1 to 65 visits with a mean of 9.1 (SD=5.6). The mean age of FEV1 measurements was 20.5 (SD=17.9), ranging from 6.0 to 68.6. FEV1pp ranged from 14.8 to 136.5 with a mean of 73.5 (SD=25.0). Although local area regression showed a decline in lung function with age, FEV1pp was highly variable at all ages, and average values at older ages showed no age-related pattern because of mortality attrition (Figure 1). The age-specific survival estimates used for mortality adjustment are provided as supplemental information; there was minimal mortality in children (>90% survival under age 18 years) but survival estimates declined steeply throughout the twenties and roughly logarithmically after age 30. The dramatic effect of adjusting for mortality attrition can be seen in Figure 2 which shows the distribution of NoRMA scores across the age range.

To illustrate the impact of these steps in calculating the severity scores, we compare two patients, a 10-year-old and a 30-year-old each with FEV1 of 60% of predicted (Table 3). The 10-year-old had an age-specific percentile score (based on residuals from the LOESS curve) of 0.10 and the 30-year-old had a score of 0.52. Adjusting for mortality attrition further improves the score for the 30-year old who is seen to have relatively mild CF lung disease while the 10-year-old is severely affected.

The two phenotypes based on mixed model analysis^{5, 15} were compared to NoRMA in the CGS sample (1435 unrelated patients). AgeDied%ile was normally distributed with a mean of 0.50 (SD=0.29). FEVest20 ranged from -31.95% to 135.84% with a mean of 72.56 (SD=20.21). Fifteen patients had FEVest20 less than 15% while four patients had estimates over 130%. Correlation coefficients for NoRMA, AgeDied%ile and FEVest20 (Table 4)

were higher in the younger subset, reflecting the limitations of the mixed model estimates at ages further from age 20, when using only three years of data for each subject.

Generalizing beyond a population-based design (KNoRMA)

Since the GMS and TSS samples were not population based, population percentiles and the NoRMA phenotype as described above could not be computed. Age-specific CF percentiles for FEV1 developed by Kulich were used to compute KNoRMA in the three samples (Table 5).

Mean age differed significantly across sites (ANOVA $P<0.001$). The TSS sample had a younger mean age than both GCS and GMS ($P<0.001$). This is consistent with previous sibling studies, which tend to enroll younger patients than those using a population based approach²¹. Conversely, GMS had an older mean age than the other two groups ($P<0.001$), reflecting the fact that mild patients under the age of 15 years were excluded from this study. There was no significant difference in the sex distributions.

Mean KNoRMA scores of CGS and GMS were not significantly different from each other, yet both were significantly higher than TSS ($P=0.003$ and $P=0.04$, respectively). The frequency distributions of KNoRMA for each of the three groups are shown in Figure 3. The distributions in TSS and CGS are very similar, whilst the distribution in GMS was bimodal, reflecting the ascertainment of patients with extreme lung phenotypes, and the recruitment of twice as many subjects in the “mild” range of lung disease compared to those in the “severe” range.

Correlation coefficients between KNoRMA and the mixed model phenotypes in TSS and GMS are shown in Table 6. Lower correlations in TSS likely reflect the fact that the TSS sample is younger and further away from the age of 20. Results were unchanged when only one sibling from each TSS family was used in analysis (data not shown).

Heritability estimate for KNoRMA

The correlation coefficient for KNoRMA in monozygous twins was 0.79 while it was 0.53 for the dizygous twin/sib group. Thus, the heritability estimate was 0.51 (estimated 95% confidence interval: 0.28, 0.77), similar to that previously reported for the Kulich percentile alone without mortality adjustment in the TSS cohort⁴.

DISCUSSION

We used age-adjusted severity disease severity and age-specific survival estimates to define a novel framework for deriving phenotypes in CF pulmonary disease. One phenotype (NoRMA) was based on a population based sample, while the second (KNoRMA) used previously published CF-specific norms for applicability across variable study designs. These measures of disease severity can be compared across a range of ages, do not require data from a long period of follow up, and can be used across disparate populations. Accounting for attrition due to disease-related mortality removes the penalty that occurs when older patients are compared only against other survivors using simply FEV1pp or CF FEV1%ile.

The mixed model estimate of FEV1 at age 20 was also designed to adjust for disease progression and mortality attrition. However, it requires extrapolation of data for patients older or younger than 20 years of age, meaning that the further from age 20, the less reliable the estimate. Use of mixed model estimates of slope and intercept to provide estimated death age percentile is also less robust in the youngest and oldest subjects where flat regression lines may yield inappropriate estimates beyond the observed measurements. This is

particularly problematic when combining study samples whose age distributions differ. These methods were designed for use in studies with extensive longitudinal data. With limited longitudinal data or cross-sectional data, these disease severity estimates will be seriously biased for many subjects, while NoRMA and KNoRMA provide valid estimates based on specific age at time of measurement for each individual. Multiple measurements can improve precision, but a single measurement is acceptable, so that subjects need not be deleted from analysis based on arbitrary rules of data availability.

Although the ascertainment schemes of the three CF samples differed drastically, KNoRMA provided a phenotype that was comparable across the sites. An additional benefit to KNoRMA (compared to mixed model methods and NoRMA) is that the phenotype estimate for each subject is independent. With the mixed model and NoRMA, lung function scores are based on the study sample; adding patients (for example, when working in a consortium) or removing patients from the sample would affect the results of all other patients. Since KNoRMA estimates are based on CF population based reference equations, addition or removal of patients from the analysis has no effect on values for the remaining patients.

There are limitations with this process that must be considered in future studies. The Kulich percentiles were based on a cohort gathered between 1994 and 2001¹⁴. It is known that lung function has improved for CF patients since that time. Therefore, lung function scores derived using the Kulich reference equations¹⁴ were expected to be higher than those derived by LOESS in the CGS sample. However, this is a known bias, in a known direction, and we hypothesize that all patients will be affected equally and the analysis of association with genetic factors should be unbiased. The Kulich scores adjust for lung function differences between the sexes, which may affect the estimation of potential sex specific effects in genetic analysis.

Another possible limitation is our use of Canadian-based mortality rates for all samples, including the US-based study samples recruited by GMS and TSS. However, data from the US Cystic Fibrosis Foundation's registry²², from which mortality for birth cohorts from 1985 onwards could be computed, indicated no significant differences in mortality estimates to age 22 (data not shown). Therefore, we do not believe that our use of Canadian-based mortality rates introduced a significant bias. Survival estimates were calculated without accounting for sex. Given historically discordant survival between males and females, future studies with separate survival estimates may be appropriate. The assumption that all CF patients who died before a given age had worse lung disease than those who survived to that age results in a small overestimate of mortality due to lung disease but one that is probably well within the margin of error of the estimates themselves since lung disease is the primary cause of death in CF. As national CF data registries around the world become more extensive, future studies may refine the CF mortality estimates in many ways – by sex, by country, by precise age, by cause of death – or may develop parametric survival models that incorporate these factors.

The principles behind this phenotype can be applied to any disease or quantitative phenotype for which a common measure for the study subjects, compared to the overall patient population, can be identified and augmented by suitable population mortality rates. Although our application is to CF, the approach can be used for any trait that is age dependent and for which cohort survival data are available. For example, in a survey of Canadians in 2005, 4.4% of those 35 years or older reported that they had been diagnosed by a health professional with chronic obstructive pulmonary disease (COPD)²³. The main measure of disease severity in COPD is FEV1, which is predictive of other complications such as cardiovascular disease²⁴, but as lung function progressively worsens in COPD patients, mortality attrition produces a bias in the comparison of younger versus older

patients. In cross-sectional studies of FEV1 in COPD, the use of published FEV1 norms and mortality rates could be used to create a mortality adjusted phenotype which could be used to find genes that may modify the progression of COPD.

The use of a disease progression and a mortality adjusted phenotype reduces the need for stratification or additional covariates in large scale genetic and epidemiological studies, increasing the power of analysis and avoiding possible distortions. This advantage will be critical²⁵ for the identification of true genetic influences that will translate to targeted therapies for individuals with high risk genotypes. In future, the inclusion of other factors related to disease progression or mortality, such as sex or early events such as poor growth or age of first serious infection, could be incorporated into the severity score, to provide a more refined measure to investigate prognosis and treatment.

Appendix

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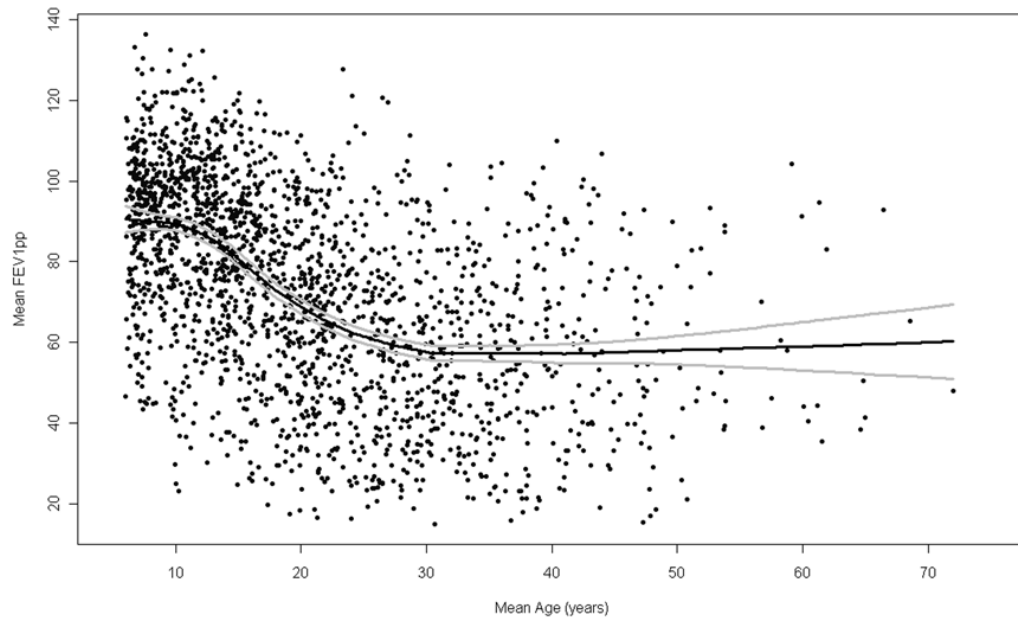


Figure 1. Cross-sectional plot of mean FEV1pp by mean age for 2061 CGS patients. Non-linear smoothed regression is plotted with 2 standard errors of the mean. Mean is based on the mean value of three years of data preceding study enrollment.

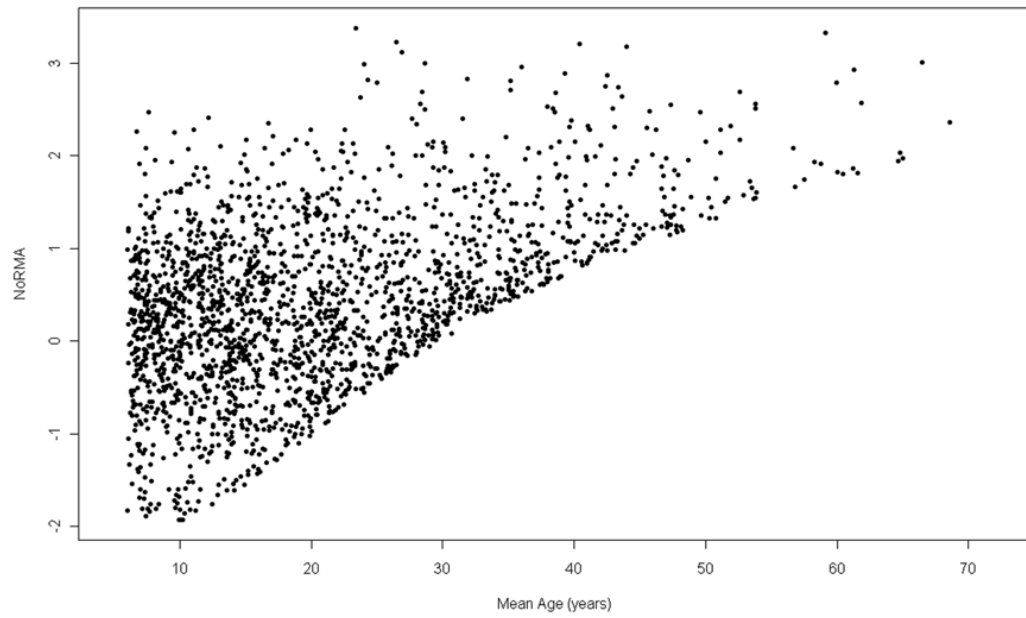


Figure 2.
Cross-sectional plot of NoRMA score by mean age for 2061 CGS patients.

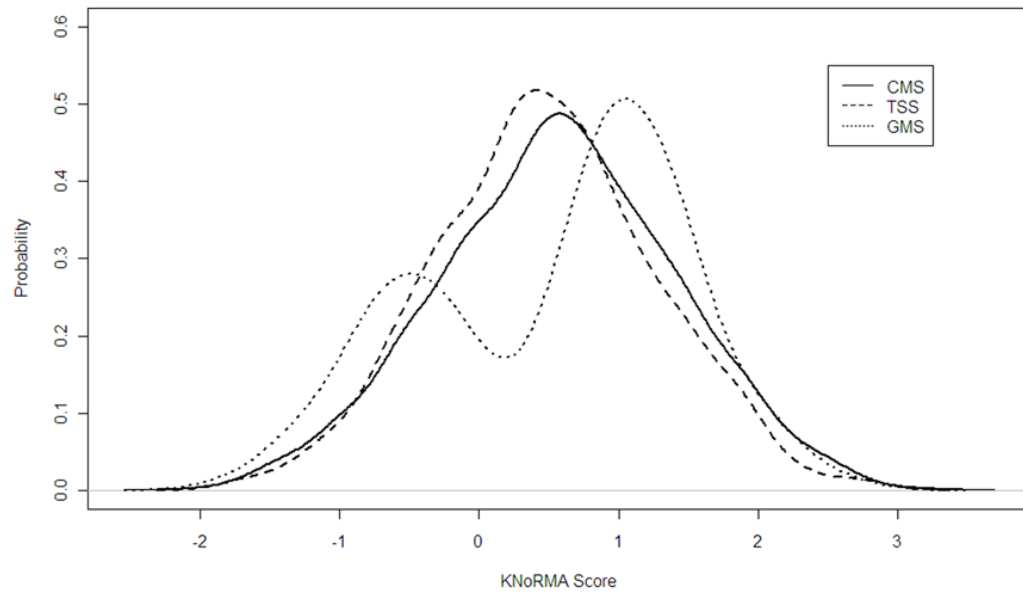


Figure 3. Frequency distributions of KNoRMA in the three study samples: 1137 GMS patients, 1323 TSS patients, and 2061 CGS patients.

Table 1

Generic Framework on Which the FEV1-Based Phenotype was Created.

1	Calculate age-specific FEV1 percentile score (X) for each patient, using either residuals from a sample based regression (as was done for NoRMA) or published disease specific reference equations (as in KNoRMA).
2	Estimate cohort survival percentile for each age in 0.01 year intervals (S).
3	Calculate age-specific mortality percentile (1-S), to adjust FEV1 percentile.
4	Calculate age-specific, survival adjusted FEV1 percentile: $X*S+(1-S)$.
5	Transform the age-specific survival adjusted FEV1 percentile to a normal score for the final phenotype.

Table 2

Comparison of CGS Sample to the Overall Canadian CF Population.

	CGS Sample (subset of CPDR)	CPDR
Number of Patients	2473	3500
Age (Mean)	19.6 ^a	18.8
Sex, %male	53.4%	52.4%
FEV1pp (Mean)	72.2	74.1
%Pancreatic Insufficient	88%	Not reported ^b
%F508del/F508del ^c	52.4%	52.5%
%F508del/Other	37.2%	35.9%

^aPatients under 2 years of age were excluded from the CGS sample producing a slightly higher mean.

^bAlthough not included in the CPDR report, the prevalence of 88% for PI is consistent with previous reports²³.

^cF508del is the most common CF-causing mutation.

Table 3

Effect of NoRMA Calculations in Two Patients (10 Year and 30 Years of Age) – With the Same FEV1pp Values.

	Younger Patient	Older Patient
Age	10 years	30 years
FEV1pp	60	60
Age-specific FEV1pp Percentile	0.10	0.52
Estimated Survival Percentile	0.97	0.49
Survival Adjusted FEV1 Percentile	0.12	0.76
NoRMA	-1.14	0.71

Table 4

Correlation Coefficients for Three Measures of Lung Disease Severity in 1435 Unrelated CGS Patients (Upper Section) and in a Subset of 917 Unrelated CGS Patients Aged 10-30 Years (Lower Section).

	CGS Patients Aged 6-50 Years (n = 1435)	NoRMA	AgeDied%ile	FEVest20
CGS Patients Aged 10-30 Years (n = 917)				
NoRMA			0.85	0.85
AgeDied%ile		0.90		0.94
FEVest20		0.91	0.94	

Table 5

Comparison of Patient Characteristics in the CGS, GMS, and TSS Samples.

	CGS	GMS	TSS
Number of Patients	2061	1137	1323
Age, mean (SD)	20.5 (11.4)	23.1 (10.2)	16.3 (9.1)
Sex, % male	53	53	52
%F508del/F508del	53.0	100	55.5
Number of useable visits per patient, mean (SD)	9.1 (5.6)	15.2 (11.9)	14.4 (9.8)
KNoRMA, mean (SD)	0.56 (0.85)	0.54 (0.96)	0.47 (0.79)

Table 6

Correlation Coefficients Between Three Measures of Lung Disease Severity in 1323 TSS Patients (Upper Section) and 1137 GMS Patients (Lower Section).

	TSS Patients (n = 1323)	KNoRMA	AgeDied%ile	FEVest20
GMS Patients (n=1137)				
KNoRMA			0.67	0.57
AgeDied%ile		0.79		0.87
FEVest20		0.81	0.89	