EUROPEAN RESPIRATORY journal
FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Original research article

Forskolin-induced Organoid Swelling is Associated with Long-term CF Disease Progression

Danya Muilwijk, Eyleen de Poel, Peter van Mourik, Sylvia W.F. Suen, Annelotte M. Vonk, Jesse E. Brunsveld, Evelien Kruisselbrink, Hugo Oppelaar, Marne C. Hagemeijer, Gitte Berkers, Karin M. de Winter-de Groot, Sabine Heida-Michel, Stephan R. Jans, Hannah van Panhuis, Menno M. van der Eerden, Renske van der Meer, Jolt Roukema, Edward Dompeling, Els. J.M. Weersink, Gerard H. Koppelman, Robert Vries, Domenique D. Zomer-van Ommen, Marinus .J.C. Eijkemans, Cornelis K. van der Ent, Jeffrey M. Beekman

Please cite this article as: Muilwijk D, de Poel E, van Mourik P, *et al*. Forskolin-induced Organoid Swelling is Associated with Long-term CF Disease Progression. *Eur Respir J* 2022; in press (https://doi.org/10.1183/13993003.00508-2021).

Forskolin-induced Organoid Swelling is Associated with Long-term CF Disease Progression

Authors:

Danya Muilwijk[1]*, Eyleen de Poel[1,2]*, Peter van Mourik[1], Sylvia W.F. Suen[1,2], Annelotte M. Vonk[1,2], Jesse E. Brunsveld[1,2], Evelien Kruisselbrink[1,2], Hugo Oppelaar[1,2], Marne C. Hagemeijer[1,2,3], Gitte Berkers[1], Karin M. de Winter-de Groot[1], Sabine Heida-Michel [1], Stephan R. Jans[1], Hannah van Panhuis [1], Menno M. van der Eerden[4], Renske van der Meer[5] , Jolt Roukema[6], Edward Dompeling[7], Els .J.M. Weersink[8], Gerard H. Koppelman[9a-b], Robert Vries[10], Domenique D. Zomer-van Ommen[11], Marinus .J.C. Eijkemans[12], Cornelis K. van der Ent[1]† and Jeffrey M. Beekman[1,2]†.

Affiliations:

[1]Department of Pediatric Respiratory Medicine, Wilhelmina Children's Hospital, University Medical Center, Utrecht University, 3584 EA Utrecht, The Netherlands;

[2]Regenerative Medicine Utrecht, University Medical Center, Utrecht University, 3584 CT Utrecht, The Netherlands;

[3]Current affiliation: Center for Lysosomal and Metabolic Diseases, Department of Clinical Genetics, Erasmus University Medical Center, 3015 GD, Rotterdam, The Netherlands;

[4]Department of Pulmonology, Erasmus MC, University Medical Center, 3015 GD Rotterdam, The Netherlands;

[5]Haga Teaching Hospital, 2545 CH The Hague, The Netherlands;

[6]Radboud University Medical Center, Radboud Institute for Health Sciences, 6525 XZ Nijmegen, The Netherlands;

[7]Maastricht University Medical Center, 6229 HX Maastricht, The Netherlands;

[8]Amsterdam University Medical Center, location AMC, 1105 AZ Amsterdam, The Netherlands;

[9a]University of Groningen, University Medical Center Groningen, Beatrix Children's Hospital, Department of Pediatric Pulmonology and Pediatric Allergology, Groningen, The Netherlands;

[9b]University of Groningen, University Medical Center Groningen, Groningen Research Institute for Asthma and COPD (GRIAC), Groningen, The Netherlands;

[10]Hubrecht Organoid Technology (HUB), 3584 CM Utrecht, The Netherlands;

[11]Dutch Cystic Fibrosis Foundation (NCFS), 3744 MG Baarn, The Netherlands;

[12]Department of Data Science and Biostatistics, Julius Center for Health Sciences and Primary Care, University Medical Center, Utrecht University, 3584 EA Utrecht, The Netherlands.

* These authors contributed equally to this work

† These authors contributed equally to this work and are both corresponding authors.

Email: J.Beekman@umcutrecht.nl (lead contact)

Take home message: Forskolin-induced swelling of patient-derived intestinal organoids is associated with long-term cystic fibrosis disease progression, expressed as FEV1pp decline and development of pancreatic insufficiency, CF-related liver disease and CF-related diabetes.

ABSTRACT

Rationale: Cystic fibrosis (CF) is a monogenic life-shortening disease associated with highly variable individual disease progression which is difficult to predict. Here we assessed the association of forskolin-induced swelling (FIS) of patient-derived organoids (PDO) with long-term CF disease progression in multiple organs and compared FIS with the golden standard biomarker sweat chloride concentration (SCC).

Methods: We retrieved 9-year longitudinal clinical data from the Dutch CF Registry of 173 people with mutations in the *cystic fibrosis transmembrane conductance regulator* (*CFTR*) gene. Individual CFTR function was defined by FIS, measured as the relative size increase of intestinal organoids after stimulation with 0.8 μM forskolin, quantified as area under the curve (AUC). We used linear mixed effect models and multivariable logistic regression to estimate the association of FIS with long-term FEV1pp decline and development of pancreatic insufficiency, CF-related liver disease and diabetes. Within these models, FIS was compared with SCC.

Results: FIS was strongly associated with longitudinal changes of lung function, with an estimated difference in annual FEV1pp decline of 0.32% (95%CI: 0.11%–0.54%; p=0.004) per 1000-points change in AUC. Moreover, increasing FIS levels were associated with lower odds of developing pancreatic insufficiency (adjusted OR: 0.18, 95%CI: 0.07–0.46, p<0.001), CF-related liver disease (adjusted OR: 0.18, 95%CI: 0.06–0.54, p=0.002) and diabetes (adjusted OR: 0.34, 95%CI: 0.12–0.97, p=0.044). These associations were absent for SCC.

Conclusion: This study exemplifies the prognostic value of a PDO-based biomarker within a clinical setting, which is especially important for people carrying rare *CFTR* mutations with unclear clinical consequences.

INTRODUCTION

Clinical disease expression in people with CF (pwCF) is variable and results from a combination of genetic, environmental and stochastic factors which are unique for each individual. CF is a recessive, monogenic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene [1]. Over 2000 *CFTR* variants which differentially affect CFTR function and clinical phenotype have been identified until now (http://cftr2.org). The more common mutations have been categorized into distinct classes according to the mechanism by which CFTR function is disrupted [2]. To better understand how CFTR function contributes to disease expression, biomarkers such as sweat chloride concentration (SCC), intestinal current measurements (ICM) and nasal potential difference (NPD) are used to estimate individual CFTR function. These biomarkers have mostly been validated in the context of CF diagnosis, but their ability to accurately discriminate between pwCF with differential disease progression is limited despite clear relations at population level [3–9]. Forskolin-induced swelling (FIS) of patient-derived intestinal organoids is an in vitro biomarker that quantifies CFTR-dependent fluid transport into the organoid lumen [10, 11] and may provide a more precise and accurate estimation of CFTR function compared to other biomarkers. Small proof of concept studies showed that FIS correlates with SCC and ICM and that clinical disease phenotypes could be stratified based on FIS level [12, 13]. We hypothesized that individual CFTR function measured by FIS is associated with long-term disease progression defined by rate of FEV1pp decline and development of co-morbidities such as pancreatic insufficiency (PI), CF-related liver disease (CFRLD) and CF-related diabetes (CFRD). Such an association supports a potential role for FIS as biomarker for long-term disease progression, which is especially relevant to people with rare, uncharacterized *CFTR* genotypes or *CFTR* genotypes with varying clinical consequences.

METHODS

Study design and population

A longitudinal cohort study was conducted in Dutch people carrying mutations in the *CFTR* gene who are included in the Dutch Cystic Fibrosis Foundation Patient Registry (DCFFPR). Of all participants, intestinal organoids were generated before January 2020 and written informed consent was obtained to use their intestinal organoids and clinical data for the present study. This study was approved by the institutional review board of the University Medical Center Utrecht, The Netherlands.

Study parameters

The primary outcome variable was defined as long-term lung function decline, expressed as FEV1 percent predicted (FEV1pp), calculated according to global lung function initiative (GLI) guidelines [14]. Secondary outcome variables were occurrence of pancreatic insufficiency (PI), defined by fecal elastase < 200 μg/g, CF-related liver disease (CFRLD), defined by hepatic steatosis or cirrhosis confirmed by imaging, and occurrence of insulin-dependent CF-related diabetes (CFRD) defined by daily insulin treatment.

The primary explanatory variable of interest was forskolin induced swelling (FIS), defined by the relative size increase of intestinal organoids after 1h stimulation with 0.8 μM forskolin, quantified as area under the curve (AUC). Previous studies showed that discrimination between individual FIS responses was most optimal and correlated best with other in vitro and in vivo CFTR biomarkers when FIS was performed with 0.8 μM forskolin [11, 12]. Other included explanatory variables were: age in years at time of each lung function measurement; treatment status at time of each lung function measurement categorized as no CFTR modulator treatment, treatment with ivacaftor or with lumacaftor/ivacaftor; sex; sweat chloride concentration (SCC) in mmol/L; and genotype, categorized as class I-V or unclassified, defined by genotype class of the mildest of both mutations according to available literature (supplementary table 1 and 2). Additionally, genotypes were categorized in groups according to the combination of the following mutation types: insertion/deletion, nonsense, missense, splice and unknown.

Study procedures

Organoid measurements:

The generation of intestinal organoids from biopsies and subsequent fluid secretion assays (FIS-assays) were performed according to a previously described protocol [15]. Rectal biopsies were collected at one timepoint during the 9-year study period. The specific time-point of rectal biopsy collection varied per study participant, but was always prior to the start of modulator therapy. FIS-assays were performed between 2014 and 2020 by analysts who were blinded for genotype and clinical data. All FIS-assay experiments were conducted in duplicate and for the majority of the donors at multiple culturing time points with a maximum of 7 consecutive culture time points (n=7).

Clinical data collection:

Data on clinical study parameters were retrieved from the DCFFPR, independent of FIS-assay results. Annual best FEV1pp values between 2010 and 2018 were used to estimate lung function decline. Treatment status at the time of each lung function measurement was calculated based on start and stop dates of CFTR modulators as registered in the DCFFPR. For SCC, PI, CFRLD and CFRD, we only collected the most recent value registered before 2019 (or before CFTR modulator treatment initiation, if applicable), as repeated measurements were unavailable or inconsistently collected. For SCC, PI ,CFRLD and CFRD, data was missing in 59 (34.1%), 63 (36.4%), 5 (2.9%) and 3 (1.7%) participants, respectively. SCC values were mostly missing for older participants, which may have been performed years before start of the registry in 2010 and were not archived within the local CF centers.

Statistical analysis

The association between age and long-term lung function decline was analyzed using a linear mixed effects model. FEV1pp was specified as outcome variable in the model, with FIS, SCC, genotype class (reference category: unclassified), sex (reference category: male), age, CFTR modulator treatment (reference category: none) and FIS*age as fixed effects, where the interaction term FIS*age reflected the difference in annual FEV1pp decline by FIS level. The model included a random intercept and random slope for age per subject,

assuming a first order auto-regressive (cAR1) correlation structure. Conditional R^2 was calculated to assess complete model performance and marginal R^2 to estimate the relative contribution of the fixed effects.

To account for selection bias towards a milder phenotype in participants surviving to an older age, a subgroup analysis was conducted including measurements between 4-25 years, in which the relationship between age and FEV1pp decline can reasonably be assumed to be linear in this dataset (figure 2a).
Sensitivity analyses were performed using genotype group, defined by the combination of mutation types, e.g. insertion/deletion, nonsense, missense, splice, unknown. Genotype group was used instead of genotype class, to assess whether the association of FIS with FEV1pp decline was influenced by categorization of genotype. To obtain reliable effect estimates and standard errors for genotype group, groups with less than 5 participants were excluded from this part of the analysis.

To compare the association of long-term FEV1pp decline with FIS versus SCC, four models were built which all included FIS, SCC, genotype class, sex, age and treatment as fixed effects. A baseline model was built without any interaction term, and the other three models were built with the addition of either the interaction term FIS*age, SCC*age or both FIS*age and SCC*age in the model. Performance of these models was compared using the Likelihood Ratio test.

Multilevel multiple imputation based on the method of chained equations [16] was used to handle missing SCC data in the linear mixed effects models. All analyses were performed on 20 imputed datasets (m=20, iterations=20) with pooling of the results.

Secondary outcomes were analyzed using multivariable logistic regression, with FIS, SCC, sex, and age at the last study measurement as explanatory variables. Given the low proportion of outcome events within some of the genotype classes as well as within genotype groups (defined by the combination of the mutation types on both alleles), genotype could not be included in these analyses. In addition, CFTR modulator usage was not included as we only collected most recent values of PI, CFRLD and CFRD before modulator initiation.
Nagelkerke's R^2 was calculated to assess model performance.

Single level multiple imputation [16] was used to handle missing data of SCC, PI and CFRD in the logistic regression models. The analyses were performed on 20 imputed datasets (m=20, iterations=20) with pooling of the results.

Significance levels were set at 0.05. All statistical analyses were performed with R version 4.1.1 using packages mice, micemd, nlme and lme4 in combination with the performance package.

RESULTS

Participant characteristics

In total, 173 participants carrying different *CFTR* genotypes provided written informed consent to collect intestinal organoid data and retrieve their clinical data from the DCFFPR. Participant characteristics are summarized in table 1. Three participants were excluded from the analysis because clinical data was not available. No data was excluded based on organoid measurements. Classification per mutation, individual genotypes with corresponding mutation classification and mutation group are listed in supplementary table 1 and 2, respectively.

Individual FIS responses

Individual FIS responses after 1 hour of stimulation with different forskolin concentrations are shown for all participants in figure 1a. Between-subject variability was most apparent at 0.8 µM and 5.0 µM forskolin, but no evident clustering was observed. Consistent with prior studies investigating relations between FIS and CF disease or biomarkers [11, 12, 17], our analyses were performed with FIS levels upon 0.8 µM forskolin stimulation. FIS data at 0.8 µM forskolin was skewed and highly variable among participants (median AUC 141.3, IQR: 30.3 – 1176.3 AUC, range: -268.0 – 4508.8 AUC; figure 1a and supplementary figure 1a) as well as within genotype classes (figure 1b-c) and between genotype groups, defined by the combination of the two mutation types (supplementary figure 1b). As expected, most organoid cultures that showed residual CFTR function (AUC>750) expressed genotypes belonging to class III-V (figure 1c). Surprisingly however, seven organoid cultures expressing genotypes categorized as class II mutation, a class for which no residual organoid

swelling upon stimulation with 0.8 µM for one hour has been previously reported [11–13], exhibited moderate to high organoid swelling (figure 1b).

Association of long-term FEV1pp decline and FIS

In total, 1054 observations of 149 participants with available FEV1pp measurements (figure 2a) were included in the analysis to assess the association of FIS with long-term FEV1pp decline. Linear mixed model analysis showed that average FEV1pp decline per year of age varied with FIS level, adjusted for sex, genotype class, CFTR modulator usage and SCC (table 2). To illustrate this association of FEV1pp decline by age with FIS, figure 2b shows that average annual FEV1pp – decline was -1.16% (95% CI: -1.43% – -0.88%; p<0.001) per year of age for participants with a FIS level of 0. Per 1000-points increase in AUC, FEV1pp decline was 0.32% (95% CI: 0.11% – 0.54%; p=0.004) per year of age lower, leading to a very mild estimated FEV1pp decline of only -0.19% per year for participants with an AUC of 3000. Model performance was excellent based on a pooled conditional R^2 of 0.979 (pooled marginal R^2 = 0.179).

The validity of these results was verified by assessing the potential impact of selection bias and confounding with separate subgroup and sensitivity analyses. A subgroup analysis in participants between 4-25 years showed a slightly higher average annual FEV1pp decline compared to the complete population (-1.57% per year (95% CI: -2.03% – -1.10%, p<0.001). Similar to the analysis in the complete cohort, FEV1pp decline varied by FIS level with 0.49% (95% CI: 0.03-0.96, p=0.039; supplementary table 3 and supplementary figure 2) per 1000-points change in AUC, suggesting a negligible impact of selection bias due to inclusion of people with *CFTR* mutations who have a milder phenotype and survive to an older age. Since at least one *CFTR* mutation was unclassified in 13.3% of participants (figure 1c, table 1 and supplementary table 1 and 2), a sensitivity analysis was performed in which we refitted both models with genotype group instead of genotype class, to assess whether the association of FIS with FEV1pp decline was influenced by categorization of genotype. The association of FIS with FEV1pp decline in these models was still statistically significant, comparable to the models categorizing genotype by mutation class (supplementary table 4).

In addition, we compared the association of FIS with FEV1pp decline versus SCC with FEV1pp decline in similar linear mixed models. SCC alone was not significantly associated with FEV1pp decline (p=0.121; supplementary table 5). An association with SCC was also absent (p=0.995, supplementary table 6) when combined with FIS in the model, suggesting a stronger association of FIS with FEV1pp decline compared to SCC. These results,

however, should be interpreted with caution due to the proportion of missing SCC data and the use of multiple imputation.

Association of CF-related co-morbidities and FIS

To investigate the association of FIS with the occurrence of other CF-related co-morbidities, we performed multivariable logistic regression with PI, CFRLD and CFRD, adjusted for age, sex and SCC. We found a significant association of FIS with the occurrence of PI (adjusted OR: 0.18, 95% CI: 0.07 – 0.46, p<0.001, Nagelkerke's R^2 = 0.496), CFRLD (adjusted OR: 0.18, 95% CI: 0.06 – 0.54, p=0.002, Nagelkerke's R^2 = 0.222) and CFRD (adjusted OR: 0.34, 95% CI: 0.12 – 0.97, p=0.044, Nagelkerke's R^2 = 0.195; table 3 and figure 3a-d). This indicates that the odds was on average 5-fold lower for developing PI and CFRLD and 3-fold lower for developing CFRD per 1000 point increase in FIS level. As illustrated in table 3 and figure 3d, age was also significantly associated with the odds of developing CFRD (adjusted OR 1.05, 95% CI: 1.02 – 1.08, p=0.004).

In combination with FIS, SCC was not associated with any of the CF-related co-morbidities, given the non-significant odds ratios of 1 (table 3). Even though multiple imputation of SCC may have influenced the strength of the associations, these results suggest that FIS is stronger associated with CF-related co-morbidities than SCC when comparing both biomarkers within the same model.

DISCUSSION

This study shows that residual CFTR function quantified by FIS of patient-derived cystic fibrosis organoids is associated with long-term annual FEV1pp decline and odds of developing CF-related co-morbidities PI, CFRLD and CFRD, using 9-year longitudinal data of Dutch people with many distinct *CFTR* mutations and ages ranging from 0 to 61 years old.

Despite the influence of genetic modifiers and other non-CFTR dependent environmental factors on CF disease severity [1, 18–20], it was remarkable to observe that in vitro FIS on intestinal cells has such a broad association with many non-intestinal organ systems. It illustrates that fluid secretion properties of CFTR in intestinal organoids are reflective of or related to CFTR function across many tissues.

As this study aimed to characterize in vitro CFTR function of many different common and rare *CFTR* mutations

with FIS, the distribution of genotypes in our dataset does not correspond to the distribution of genotypes typical for the Dutch population, in which F508del/F508del is the most common genotype. Yet it improves generalizability of our results to the population with rare *CFTR* mutations for which this study is especially relevant. In addition, rectal biopsies of the participants that have received modulator therapy were collected prior to the start of modulator therapy, so intestinal organoid measurements were not influenced by treatment.

Direct comparison of FIS with SCC revealed that FIS was stronger associated with long-term multi-organ disease expression compared to SCC, which has been the most important and well-validated biomarker of CF disease until now and is a commonly used endpoint to measure efficacy of CFTR modulating drugs [5, 6]. Although the association with SCC could have been influenced by missing values and type of imputation method, the difference between FIS and SCC might also be explained by a more precise and accurate estimation of CFTR function by FIS. FIS facilitates repeated measures and is completely CFTR dependent, which reduces the impact of technological and non-CFTR biological variability in the in vitro assay [10, 11], whereas a substantial part of variability in SCC is caused by technical and other non-CFTR dependent biological factors [5]. Additional studies with complete datasets including repeated measurements for more precise typing of SCC are required to confirm these findings. Alternatively, it would be interesting to explore if novel sweat-based readouts that may show a higher dependency on CFTR function might also lead to better correlations with clinical observations.

In addition, FIS could be compared with other biomarkers that are being used for CF diagnosis, such as NPD and ICM. Although NPD has been used to discriminate between non-CF and CF [3, 4, 6–9] its ability to accurately discriminate between pwCF with differential disease progression is limited. While ICM measurements are more sensitive and have a larger dynamic range than NPD, generation of a large dataset with repeated measures is hampered by the need for fresh rectal biopsies.

The data also suggested that FIS has additional value in the context of disease severity association beyond the current CFTR mutation classification system. For our statistical models, we needed to prioritize one particular mutational subclass for each CFTR mutation, which is difficult due to lack of detailed experimental data for many rare (missense) mutations and the impact of potential multiple mechanistic defects for single mutations [21]. This complicates studies between mutation classification and relation with disease severity. CFTR function by FIS demonstrated a large variability in CFTR function between participants with different genotypes but also

within genotype classes. FIS may thus have the potential to help to further refine patient-based classification systems beyond current genotype classification models. This might lead to more precise individual typing and prediction of disease, compared to the current classification of 'mild' and 'severe' CF phenotypes [22–24] or the CFTR2 based classification of mutations (CF-causing, varying clinical consequences, non-CF causing).

Rates of annual FEV1pp decline in this study were within the same range as reported by other recent European studies, which also showed that annual FEV1pp decline was lower for pwCF with a 'milder' disease severity as classified by genotype [25] or pancreatic status [26] and was highest in the age group between 18 and 28 years [26]. Moreover, our results are consistent with a previous study showing a more severe CF disease phenotype in terms of pulmonary and gastro-intestinal outcome parameters in infants with low FIS compared to infants with high FIS [12]. In line with our observations, Davis et al. also demonstrated that SCC by itself does not predict lung disease in pwCF [27].

In addition to the relation of FIS with disease severity, several studies already showed that average FIS response to CFTR modulators was also correlated with short-term clinical drug response across groups with different genotypes [11, 17] and in individuals with a variety of CFTR mutations [28]. On the other hand, different exploratory studies did not detect an association of FIS with short-term clinical response to lumacaftor/ivacaftor in pwCF homozygous for F508del [29] or heterozygous for the A455E mutation [30] or to ivacaftor in people with residual CFTR-function mutations [31]. These studies also did not demonstrate associations between FIS and biomarkers of CFTR function (NPD, SCC and ICM) [29] or FIS and SCC [30, 31], and no relations between any biomarker of CFTR function and clinical response. Also, treatment magnitude at group level was absent [29, 30] or limited [31], suggesting that the relative impact of CFTR-dependent factors over non-CFTR dependent factors to between-patient variations was lower as compared to the study of Berkers et al [28]. This generally lowers the ability of FIS or any individual outcome to correlate after a CFTR modulator treatment. Further research in larger study populations is therefore warranted to study the association of changes in FIS or other biomarkers of CFTR function with long-term clinical effects upon CFTR modulator therapy in homogeneous and heterogeneous populations with CF.

An important limitation of this research is the retrospective observational study design. We adjusted for several confounders, but were unable to account for other prognostic factors such as pulmonary exacerbations and sputum cultures. As 34% of SCC values was missing, we used multiple imputation methods to prevent bias

due to selective missing data, but this may still have influenced the associations with SCC and its comparison with FIS. Potential impact of survival bias was minimized by our subgroup and sensitivity analyses, but could not completely be excluded. Additional prospective studies should be performed to confirm the predictive value of FIS in comparison with other biomarkers such as SCC, NPD and ICM, yet our findings are in line with previous work that already demonstrated the potential of FIS as biomarker of CF disease.

In summary, this study showed that FIS of cystic fibrosis intestinal organoids is strongly associated with long-term FEV1pp decline and odds of developing different CF-related co-morbidities, suggesting that estimation of CFTR function by FIS could have important prognostic value for individual disease expression of multiple, critical organs that are affected by CF.

Acknowledgements

We would like to thank all participants who gave informed consent for generating and testing their individual organoids and the use of their data; all members of the research teams of the Dutch CF clinics that contributed to this work; and all colleagues of the HUB Organoid Technology for their help with generating intestinal organoids and performing FIS experiments.

Funding

This work was funded by grants of the Dutch Cystic Fibrosis Foundation (NCFS) as part of the HIT-CF Program and by ZonMW.

Author Contribution Statement

D.M. and E.d.P. contributed substantially to the design of the study, the acquisition, verification, analysis and interpretation of the data and have drafted the manuscript. S.W.F.S., A.M.V., J.E.B., E.K., H.O., M.C.H., P.v.M. G.B., K.M.d.W-d.G., S.H.-M., S.R.J., H.v.P., M.M.v.d.E., R.v.d.M., J.R., E.D., E.J.M.W., G.H.K., R.V. and D.D.Z.-v.O. contributed to the acquisition of study data and revised the manuscript. M.J.C.E. contributed to the design of the study, analysis and interpretation of data and revised the manuscript. C.K.v.d.E and J.M.B. have made

substantial contributions to the conception and design of the study, interpretation of data and revised the manuscript.

Declaration of interests

J.M.B. reports personal fees from Vertex Pharmaceuticals, Proteostasis Therapeutics, Eloxx Pharmaceuticals, Teva Pharmaceutical Industries and Galapagos, outside the submitted work; In addition, J.M.B. has a patent patent(s) related to the FIS-assay with royalties paid. C.K.v.d.E. reports grants from GSK, Nutricia, TEVA, Gilead, Vertex, ProQR, Proteostasis, Galapagos NV and Eloxx, outside the submitted work; In addition, C.K.v.d.E. has a patent 10006904 with royalties paid. G.H.K. reports grants from Lung Foundation of the Netherlands, Vertex Pharmaceuticals, UBBO EMMIUS foundation, GSK, TEVA the Netherlands, TETRI Foundation, European Union (H2020), outside the submitted work; and he has participated in advisory boards meetings to GSK and PURE-IMS outside the submitted work (Money to institution). P.v.M. reports financial compensation (money to institution) from Vertex for participation in a webinar, outside the submitted work. All other authors have nothing to disclose.

REFERENCES

1. Cutting GR. Cystic fibrosis genetics: From molecular understanding to clinical application. *Nat. Rev. Genet.* 2015; 16: 45–56.

2. Elborn JS. Cystic fibrosis. *Lancet (London, England)* England; 2016; 388: 2519–2531.

3. Beekman JM, Sermet-Gaudelus I, de Boeck K, Gonska T, Derichs N, Mall MA, Mehta A, Martin U, Drumm M, Amaral MD. CFTR functional measurements in human models for diagnosis, prognosis and personalized therapy: Report on the pre-conference meeting to the 11th ECFS Basic Science Conference, Malta, 26-29 March 2014. *J. Cyst. Fibros. Off. J. Eur. Cyst. Fibros. Soc.* Netherlands; 2014; 13: 363–372.

4. Bronsveld I, Mekus F, Bijman J, Ballmann M, Greipel J, Hundrieser J, Halley DJ, Laabs U, Busche R, De Jonge HR, Tümmler B, Veeze HJ. Residual chloride secretion in intestinal tissue of deltaF508 homozygous twins and siblings with cystic fibrosis. The European CF Twin and Sibling Study Consortium. *Gastroenterology* United States; 2000; 119: 32–40.

5. Collaco JM, Blackman SM, Raraigh KS, Corvol H, Rommens JM, Pace RG, Boelle P-Y, McGready J, Sosnay PR, Strug LJ, Knowles MR, Cutting GR. Sources of Variation in Sweat Chloride Measurements in Cystic Fibrosis. *Am. J. Respir. Crit. Care Med.* 2016; 194: 1375–1382.

6. De Boeck K, Kent L, Davies J, Derichs N, Amaral M, Rowe SM, Middleton P, de Jonge H, Bronsveld I, Wilschanski M, Melotti P, Danner-Boucher I, Boerner S, Fajac I, Southern K, de Nooijer RA, Bot A, de Rijke Y, de Wachter E, Leal T, Vermeulen F, Hug MJ, Rault G, Nguyen-Khoa T, Barreto C, Proesmans M, Sermet-Gaudelus I. CFTR biomarkers: time for promotion to surrogate end-point. *Eur. Respir. J.* England; 2013; 41: 203–216.

7. Kyrilli S, Henry T, Wilschanski M, Fajac I, Davies JC, Jais J-P, Sermet-Gaudelus I. Insights into the variability of nasal potential difference, a biomarker of CFTR activity. *J. Cyst. Fibros. Off. J. Eur. Cyst. Fibros. Soc.* Netherlands; 2020; 19: 620–626.

8. Mall M, Kreda SM, Mengos A, Jensen TJ, Hirtz S, Seydewitz HH, Yankaskas J, Kunzelmann K, Riordan JR, Boucher RC. The DeltaF508 mutation results in loss of CFTR function and mature protein in native

human colon. *Gastroenterology* United States; 2004; 126: 32–41.

9. Minso R, Schulz A, Dopfer C, Alfeis N, Barneveld A van, Makartian-Gyulumyan L, Hansen G, Junge S, Müller C, Ringshausen FCC, Sauer-Heilborn A, Stanke F, Stolpe C, Tamm S, Welte T, Dittrich A-M, Tümmler B. Intestinal current measurement and nasal potential difference to make a diagnosis of cases with inconclusive CFTR genetics and sweat test. *BMJ open Respir. Res.* 2020; 7.

10. Dekkers JF, Wiegerinck CL, de Jonge HR, Bronsveld I, Janssens HM, de Winter-de Groot KM, Brandsma AM, de Jong NWM, Bijvelds MJC, Scholte BJ, Nieuwenhuis EES, van den Brink S, Clevers H, van der Ent CK, Middendorp S, Beekman JM. A functional CFTR assay using primary cystic fibrosis intestinal organoids. *Nat. Med.* United States; 2013; 19: 939–945.

11. Dekkers JF, Berkers G, Kruisselbrink E, Vonk A, de Jonge HR, Janssens HM, Bronsveld I, van de Graaf EA, Nieuwenhuis EES, Houwen RHJ, Vleggaar FP, Escher JC, de Rijke YB, Majoor CJ, Heijerman HGM, de Winter-de Groot KM, Clevers H, van der Ent CK, Beekman JM. Characterizing responses to CFTR-modulating drugs using rectal organoids derived from subjects with cystic fibrosis. *Sci. Transl. Med.* United States; 2016; 8: 344ra84.

12. de Winter-de Groot KM, Janssens HM, van Uum RT, Dekkers JF, Berkers G, Vonk A, Kruisselbrink E, Oppelaar H, Vries R, Clevers H, Houwen RHJ, Escher JC, Elias SG, de Jonge HR, de Rijke YB, Tiddens HAWM, van der Ent CK, Beekman JM. Stratifying infants with cystic fibrosis for disease severity using intestinal organoid swelling as a biomarker of CFTR function. *Eur. Respir. J.* England; 2018; 52.

13. de Winter-de Groot KM, Berkers G, Marck-van der Wilt REP, van der Meer R, Vonk A, Dekkers JF, Geerdink M, Michel S, Kruisselbrink E, Vries R, Clevers H, Vleggaar FP, Elias SG, Heijerman HGM, van der Ent CK, Beekman JM. Forskolin-induced swelling of intestinal organoids correlates with disease severity in adults with cystic fibrosis and homozygous F508del mutations. *J. Cyst. Fibros. Off. J. Eur. Cyst. Fibros. Soc.* Netherlands; 2020; 19: 614–619.

14. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, Enright PL, Hankinson JL, Ip MSM, Zheng J, Stocks J. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur. Respir. J.* 2012; 40: 1324–1343.

15. Vonk AM, van Mourik P, Ramalho AS, Silva IAL, Statia M, Kruisselbrink E, Suen SWF, Dekkers JF, Vleggaar FP, Houwen RHJ, Mullenders J, Boj SF, Vries R, Amaral MD, de Boeck K, van der Ent CK, Beekman JM. Protocol for Application, Standardization and Validation of the Forskolin-Induced Swelling Assay in Cystic Fibrosis Human Colon Organoids. *STAR Protoc.* 2020; 1: 100019.

16. van Buuren S. Flexible Imputation of Missing Data [Internet]. 2nd editio. Chapman & Hall/CRC; 2018.Available from: https://stefvanbuuren.name/fimd/.

17. Ramalho AS, Fürstová E, Vonk AM, Ferrante M, Verfaillie C, Dupont L, Boon M, Proesmans M, Beekman JM, Sarouk I, Vazquez Cordero C, Vermeulen F, De Boeck K. Correction of CFTR function in intestinal organoids to guide treatment of cystic fibrosis. *Eur. Respir. J.* England; 2021; 57.

18. Collaco JM, Blackman SM, McGready J, Naughton KM, Cutting GR. Quantification of the relative contribution of environmental and genetic factors to variation in cystic fibrosis lung function. *J. Pediatr.* 2010; 157: 802–803.

19. Santis G, Osborne L, Knight RA, Hodson ME. Independent genetic determinants of pancreatic and pulmonary status in cystic fibrosis. *Lancet (London, England)* England; 1990; 336: 1081–1084.

20. Weiler CA, Drumm ML. Genetic influences on cystic fibrosis lung disease severity. *Front. Pharmacol.* 2013; 4: 40.

21. Veit G, Avramescu RG, Chiang AN, Houck SA, Cai Z, Peters KW, Hong JS, Pollard HB, Guggino WB, Balch WE, Skach WR, Cutting GR, Frizzell RA, Sheppard DN, Cyr DM, Sorscher EJ, Brodsky JL, Lukacs GL. From CFTR biology toward combinatorial pharmacotherapy: expanded classification of cystic fibrosis mutations. *Mol. Biol. Cell* 2016; 27: 424–433.

22. McKone EF, Emerson SS, Edwards KL, Aitken ML. Effect of genotype on phenotype and mortality in cystic fibrosis: a retrospective cohort study. *Lancet (London, England)* England; 2003; 361: 1671–1676.

23. McKone EF, Goss CH, Aitken ML. CFTR genotype as a predictor of prognosis in cystic fibrosis. *Chest* United States; 2006; 130: 1441–1447.

24. US CF Foundation, Johns Hopkins University TH for SC. The Clinical and Functional TRanslation of CFTR (CFTR2) [Internet]. 2011.Available from: http://cftr2.org.

25. De Boeck K, Zolin A. Year to year change in FEV(1) in patients with cystic fibrosis and different mutation classes. *J. Cyst. Fibros. Off. J. Eur. Cyst. Fibros. Soc.* Netherlands; 2017; 16: 239–245.

26. Caley L, Smith L, White H, Peckham DG. Average rate of lung function decline in adults with cystic fibrosis in the United Kingdom: Data from the UK CF registry. *J. Cyst. Fibros. Off. J. Eur. Cyst. Fibros. Soc.* Netherlands; 2020; .

27. Davis PB, Schluchter MD, Konstan MW. Relation of sweat chloride concentration to severity of lung disease in cystic fibrosis. *Pediatr. Pulmonol.* United States; 2004; 38: 204–209.

28. Berkers G, van Mourik P, Vonk AM, Kruisselbrink E, Dekkers JF, de Winter-de Groot KM, Arets HGM, Marck-van der Wilt REP, Dijkema JS, Vanderschuren MM, Houwen RHJ, Heijerman HGM, van de Graaf EA, Elias SG, Majoor CJ, Koppelman GH, Roukema J, Bakker M, Janssens HM, van der Meer R, Vries RGJ, Clevers HC, de Jonge HR, Beekman JM, van der Ent CK. Rectal Organoids Enable Personalized Treatment of Cystic Fibrosis. *Cell Rep.* United States; 2019; 26: 1701-1708.e3.

29. Graeber SY, van Mourik P, Vonk AM, Kruisselbrink E, Hirtz S, van der Ent CK, Mall MA, Beekman JM. Comparison of Organoid Swelling and In Vivo Biomarkers of CFTR Function to Determine Effects of Lumacaftor-Ivacaftor in Patients with Cystic Fibrosis Homozygous for the F508del Mutation. Am. J. Respir. Crit. Care Med. United States; 2020. p. 1589–1592.

30. Berkers G, van der Meer R, Heijerman H, Beekman JM, Boj SF, Vries RGJ, van Mourik P, Doyle JR, Audhya P, Yuan ZJ, Kinnman N, van der Ent CK. Lumacaftor/ivacaftor in people with cystic fibrosis with an A455E-CFTR mutation. *J. Cyst. Fibros. Off. J. Eur. Cyst. Fibros. Soc.* Netherlands; 2021; 20: 761–767.

31. Kerem E, Cohen-Cymberknoh M, Tsabari R, Wilschanski M, Reiter J, Shoseyov D, Gileles-Hillel A, Pugatsch T, Davies JC, Short C, Saunders C, DeSouza C, Sullivan JC, Doyle JR, Chandarana K, Kinnman N. Ivacaftor in People with Cystic Fibrosis and a 3849+10kb C→T or D1152H Residual Function Mutation. *Ann. Am. Thorac. Soc.* 2021; 18: 433–441.

TABLES

Table 1. Participant characteristics

N=173	
Age, median (IQR)	19.5 (9.5 – 30.5)
Sex, n (%)	
Male	87 (50.3)
Female	86 (49.7)
Mutation class, n (%)	
Class I	15 (8.7)
Class II	91 (52.5)
Class III	11 (6.4)
Class IV	10 (5.8)
Class V	23 (13.3)
Unclassified	23 (13.3)
CFTR modulator usage, n (%)	
Ivacaftor	16 (9.2)
Lumacaftor/ivacaftor	8 (4.6)
FIS, median (IQR)	141.3 (30.3 – 1176.3)
SCC, mean (SD)	92.6 (25.9)
Missing values, n (%)	59 (34.1)
FEV1pp, mean (SD)	75.9 (23.2)
Pancreatic function, n (%)	
Insufficient (fecal elastase <200 µg/g)	75 (43.4)
Sufficient (fecal elastase ≥200 µg/g)	35 (20.2)
Missing values	63 (36.4)
CF-related liver disease, n (%)	44 (25.4)
Missing values	5 (2.9)
CF-related diabetes, n (%)	25 (14.5)
Missing values	3 (1.7)

Age in years. Genotype: genotype class of the mildest of both mutations. FIS: Forskolin induced swelling, defined as the relative size increase of intestinal organoids (AUC) after 1h stimulation with 0.8 µmol/L forskolin. SCC: Sweat chloride concentration in mmol/L. FEV1pp: Forced expiratory volume in 1 second, percent predicted.

Table 2. Association of FIS with FEV1pp decline

Regression coefficients of linear mixed effects model for FEV1pp.

N=149, obs=1054	**Coefficient (95% CI)**	**P-value**
Age	-1.16 (-1.43 – -0.88)	<0.001*
FIS	-2.47 (-8.92 – 3.99)	0.454
FIS*age	0.32 (0.11 – 0.54)	0.004*
Treatment		
- none	Reference category	
- ivacaftor	7.99 (4.58 – 11.40)	<0.001*
- lumacaftor/ivacaftor	-3.83 (-8.28 – -0.62)	0.092
Sex		
- male	Reference category	
- female	-0.96 (-7.00 – 5.08)	0.754
Genotype class		
- unclassified	Reference category	
- class I	0.18 (-13.92 – 14.27)	0.980
- class II	5.13 (-5.76 – 16.01)	0.356
- class III	10.25 (-3.79 – 24.28)	0.152
- class IV	11.01 (-5.36 – 27.38)	0.187
- class V	-2.31 (-16.95 – 12.33)	0.757
SCC	-0.09 (-0.25 – 0.06)	0.239

*FEV1pp: Forced expiratory volume in 1 second, percent predicted. Age in years. FIS: Forskolin-induced swelling, defined as the relative size increase of intestinal organoids (AUC) after 1h stimulation with 0.8 µM/L forskolin, coefficient scaled 1:1000 AUC. SCC: Sweat chloride concentration in mmol/L. FIS*age indicates the difference in annual FEV1pp decline per 1000 AUC change in FIS level. Genotype class: CFTR protein function class of the mildest of both CFTR mutations. Pooled conditional $R^2 = 0.979$, marginal $R^2 = 0.179$. *Significance level $P < 0.05$.*

Table 3. Association of FIS with CF-related co-morbidities

Adjusted odds ratios of multivariable logistic regression for pancreatic insufficiency, CF-related diabetes and CF-related liver disease.

N=170	Pancreatic insufficiency		CF-related liver disease		CF-related diabetes	
	Odds ratio (95% CI)	**P-value**	**Odds ratio (95% CI)**	**P-value**	**Odds ratio (95% CI)**	**P-value**
FIS	0.18 (0.07 – 0.46)	<0.001*	0.18 (0.06 – 0.54)	0.002*	0.34 (0.12 – 0.97)	0.044*
Age	0.98 (0.93 – 1.02)	0.300	1.02 (0.99 – 1.05)	0.229	1.05 (1.02 – 1.08)	0.004*
Sex						
- male	Reference category		Reference category		Reference category	
- female	0.46 (0.14 – 1.46)	0.181	0.68 (0.32 – 1.44)	0.313	2.08 (0.81 – 5.37)	0.127
SCC	1.00 (0.97 – 1.04)	0.944	1.00 (0.98 – 1.02)	0.913	1.00 (0.97 – 1.04)	0.838

*FIS: Forskolin-induced swelling, defined as the relative size increase of intestinal organoids (AUC) after 1h stimulation with 0.8 µM forskolin, coefficients scaled 1:1000 AUC. Age in years. SCC: sweat chloride concentration in mmol/L. Nagelkerke's R^2 pancreatic insufficiency = 0.496, CF-related liver disease = 0.223, CF-related diabetes = 0.195. * Significance level P < 0.05.*

FIGURE LEGENDS

Figure 1. Forskolin-induced swelling (FIS) levels of organoids derived from the 173 study participants.

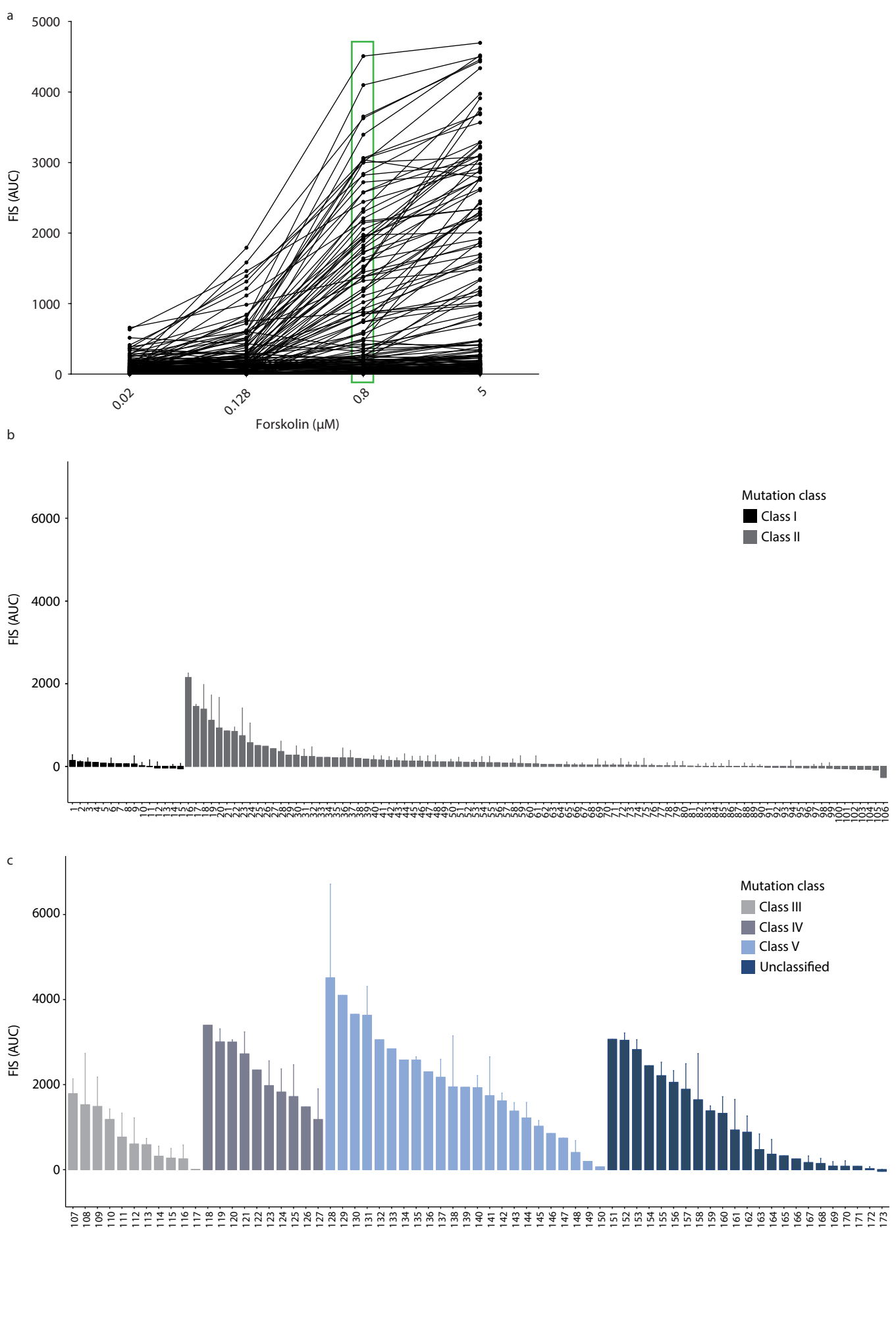
a) FIS levels, defined by relative size increase of intestinal organoids after 1h stimulation with four ascending forskolin concentrations, quantified as area under the curve (AUC). Each line represents swelling of organoids of individual study participants. Each data point (black dot) represents mean AUC of both technical (n=2) and biological replicates (ranging from n=1 to n=7). b) Waterfall plots of FIS responses at 0.8 µM forskolin (highlighted by the green box) of all study participants grouped based on mutation class I or II or c) mutation class III-V or unclassified. Genotypes are categorized into one mutation class based on the mildest mutation class of the two alleles. Bars represent mean+SD of all replicates. The numbers on the x-axes represent participant number, whereas corresponding genotypes are specified in supplementary table 2.

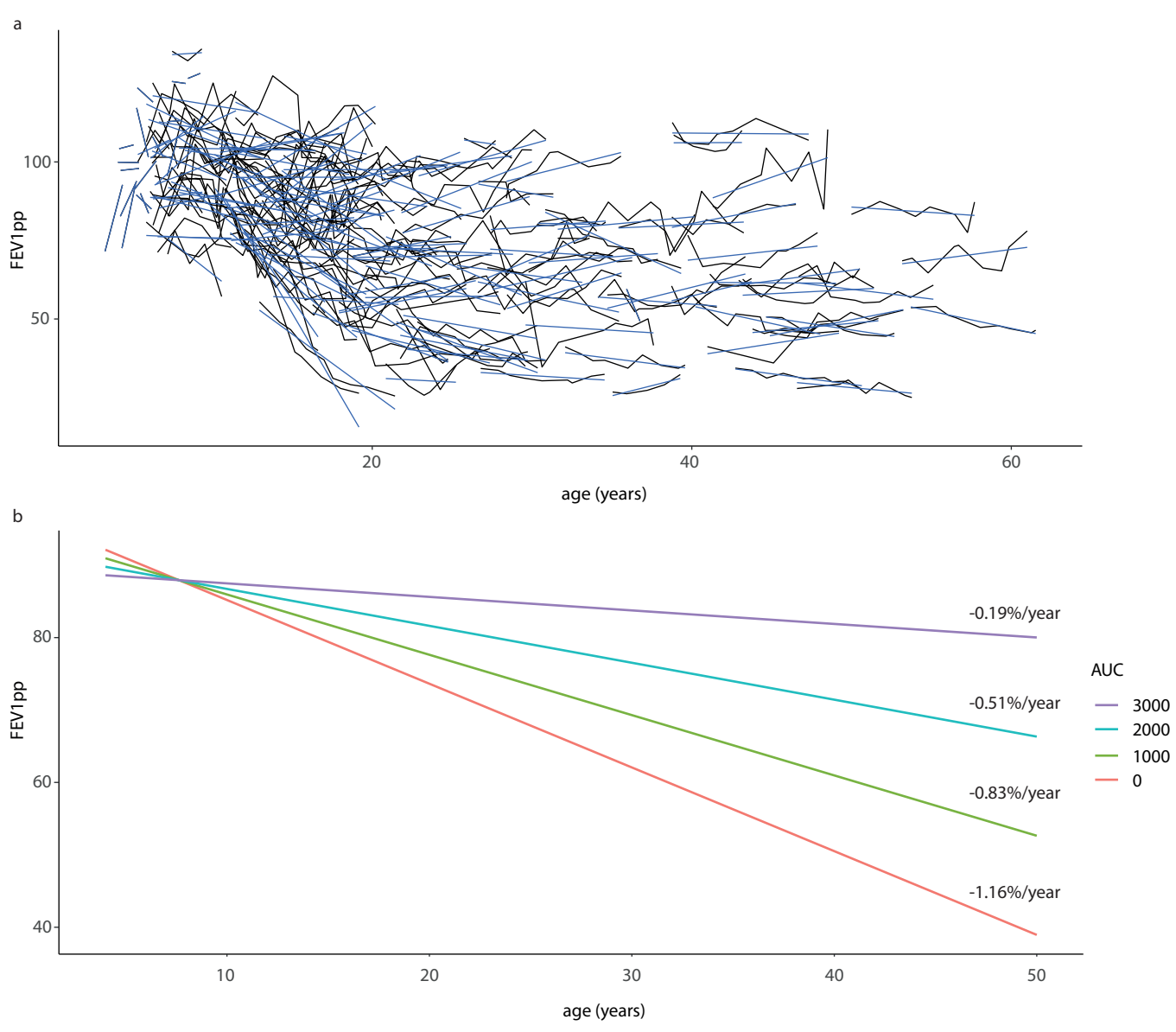
Figure 2. Association of FIS with long-term FEV1pp decline.

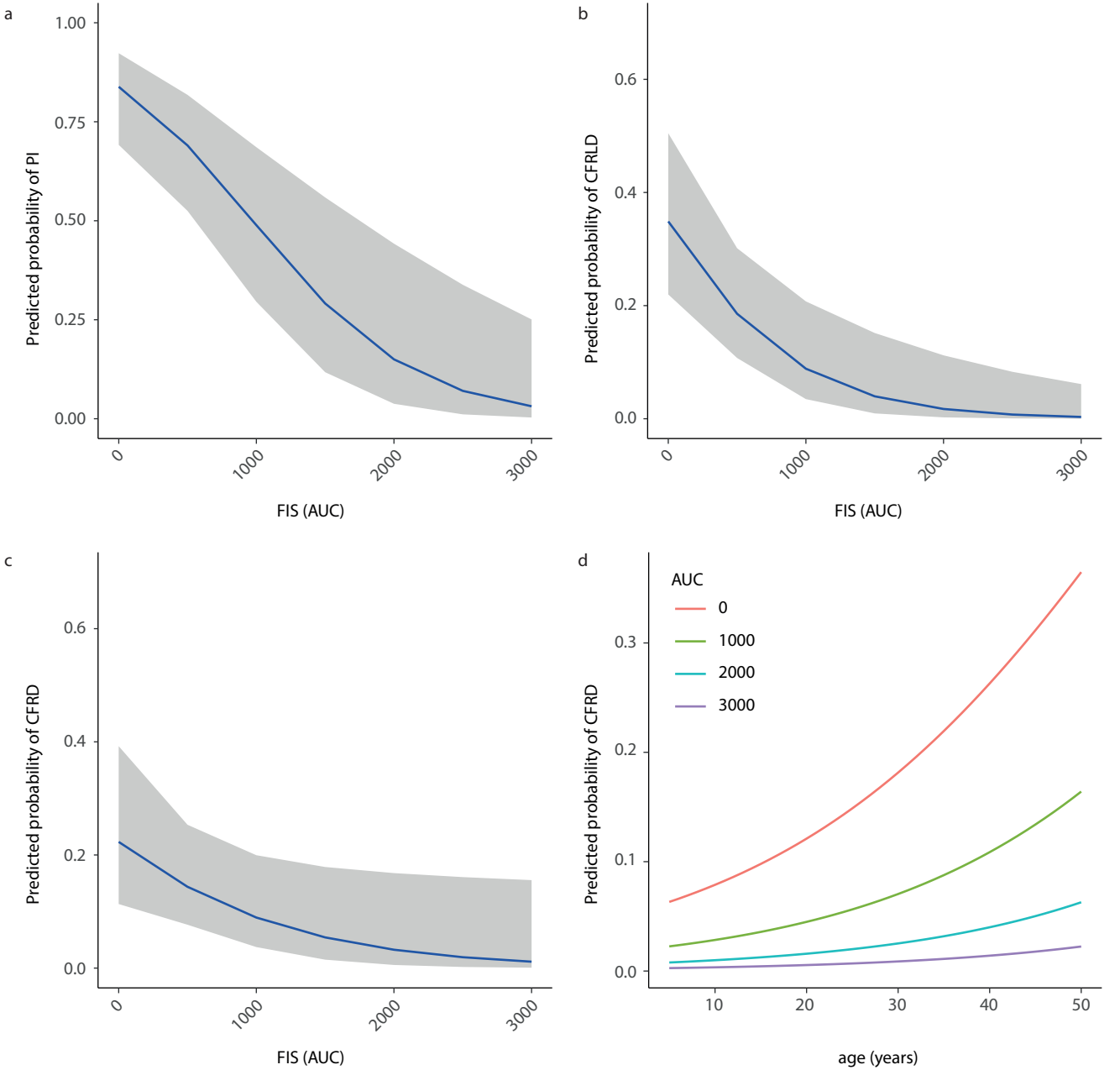
a) Individual FEV1pp trajectories of study participants over time in years. Black lines represent individual observed FEV1pp trajectories, whereas the blue lines represent estimated average annual FEV1pp slope per individual. b) Predicted FEV1pp decline based on linear mixed effects model coefficients in table 2, illustrating the association between different levels of residual CFTR function and long-term FEV1pp decline. Analysis was performed with FIS as continuous variable, yet for illustrative purposes predicted FEV1pp decline is plotted by steps of 1000 AUC. Average predicted annual FEV1pp decline per 1000 AUC is specified on the right. The lower limit of the x-axis was set at 4 years, because the feasibility and generalizability of ppFEV1 measurements is limited for younger children. Pooled conditional R^2 = 0.977, marginal R^2 = 0.179.

Figure 3. Association of FIS with CF-related comorbidities.

Association between residual CFTR function (illustrated by steps of 1000 AUC) and probability of developing pancreatic insufficiency (PI) (a), CF-related liver disease (CFRLD) (b) and CF-related diabetes (CFRD) (c). In addition to FIS, age is also associated with probability of developing CFRD (d). Nagelkerke's R^2: PI = 0. 496, CFRLD = 0.223, CFRD = 0.195.


a
5000
4000
3000
2000
1000
0
FIS (AUC)
0.02
0.128
0.8
5
Forskolin (μM)
b
6000
4000
2000
0
FIS (AUC)
Mutation class
Class I
Class II
c
6000
4000
2000
0
FIS (AUC)
Mutation class
Class III
Class IV
Class V
Unclassified


a
FEV1pp
100
50
20
40
60
age (years)
b
FEV1pp
80
60
40
10
20
30
40
50
age (years)
-0.19%/year
-0.51%/year
-0.83%/year
-1.16%/year
AUC
3000
2000
1000
0


a
Predicted probability of PI
1.00
0.75
0.50
0.25
0.00
0
1000
2000
3000
FIS (AUC)
b
Predicted probability of CFRLD
0.6
0.4
0.2
0.0
0
1000
2000
3000
FIS (AUC)
c
Predicted probability of CFRD
0.6
0.4
0.2
0.0
0
1000
2000
3000
FIS (AUC)
d
Predicted probability of CFRD
0.3
0.2
0.1
0.0
AUC
0
1000
2000
3000
10
20
30
40
50
age (years)

SUPPLEMENTARY MATERIAL

Supplementary table 1. Classification of mutations.

variant cDNA name	variant protein name	variant legacy name	Classification [REF]	mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.948delT	p.Phe316LeufsX12	1078delT	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[1]	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.1006_1007insG	p.Ile336SerfsX28	1138insG	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	CFTR1 reference [3]: Schwarz M, Malone G, Super M 1992-03-16 (reference not found on Pubmed).	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF in CFTR2.
c.1210-1delG	No protein name	1342-1delG	I	Splice	Not described in CFTR2.	CFTR 1 reference [3]: Huang Q, Yuan XW, Zielenski J 2008-07-11 (reference not found on Pubmed).	Class I CFTR protein synthesis defect based on sequence analysis (a splice site mutation that affects an invariant splice site sequence (+1,+2, -1,-2), leading to inproper splicing of the intron-exon boundary). Although no disease classification is present in CFTR2, we classify invariant splice site sequence variations as class I defects due to the critical impact of invariant splice mutations on splicing. This variant is described in CFTR1, but variant cDNA name and legacy name show no hits in Pubmed in the context of CF.
c.1211delG	p.Gly404AspfsX38	1343delG	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	Not described in CFTR1 [3].	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF [4].
c.1545_1546delTA	p.Tyr515X	1677delTA	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[5]	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF [2].

c.1585-1G>A	No protein name	1717-1G->A	I	Splice	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[6]	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.1679+1G>C	No protein name	1811+1G->C	I	Splice	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[7]	Class I CFTR protein synthesis defect based on sequence analysis (a splice site mutation that affects an invariant splice site sequence, leading to inproper splicing of the intron-exon boundary) associated with PI-CF [6].
c.1680-1G>A	No protein name	1812-1G->A	I	Splice	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[8]	Class I CFTR protein synthesis defect based on sequence analysis (a splice site mutation that affects an invariant splice site sequence, leading to inproper splicing of the intron-exon boundary) associated with PI-CF [2].
c.1681_1682insC	p.Val562SerfsX6	1813insC	I	Ins/del	Not described in CFTR2.	CFTR1 reference [3]: Scheffer H, Wu Y, Hofstra R, Looman M, Buys C 1996-10-23 (reference not found on Pubmed).	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein.
c.1766+5G>T	No protein name	1898+5G->T	V	Splice	This variant causes CF when combined with another CF-causing variant. 67% (N=4) of patients in CFTR2 who have this variant are pancreatic insufficient.	[9]	Class V splice mutation associated with reduced wild type CFTR function based on sequence analysis (intronic variant outside invariant splice site) and associated with some PS-CF [9].
c.1911delG	p.Gln637HisfsX26	2043delG	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[10]	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF in CFTR2.
c.2051_2052delAAinsG	p.Lys684SerfsX38	2183AA->G	I	ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	CFTR1 reference [3]: Leoni GB, Rosatelli MC, Cao A 1994-01-13 (reference not found on pubmed).	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF [2, 11].

c.2052delA	p.Lys684Asnfs X38	2184delA	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[12]	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.2052_2053insA	p.Gln685Thrfs X4	2184insA	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	CFTR1 reference [3]: Kalin N, Dork T, Tummler B 1992-01-02 (reference not found on Pubmed).	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF [2, 13].
c.2657+5G>A	No protein name	2789+5G->A	V	Splice	This variant causes CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	[14]	Class V splice mutation associated with reduced wild type CFTR function based on sequence analysis (intronic variant outside invariant splice site) and associated with PS-CF [2, 14].
c.2988+1G>A	No protein name	3120+1G->A	I	Splice	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[15]	Class I CFTR protein synthesis defect based on sequence analysis (a splice site mutation that affects an invariant splice site sequence, leading to inproper splicing of the intron-exon boundary) associated with PI-CF [2].
c.3140-26A>G	No protein name	3272-26A->G	V	Splice	This variant causes CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	[10]	Class V splice mutation associated with reduced wild type CFTR function based on sequence analysis (intronic variant outside invariant splice site) and associated with PS-CF [2].
c.233_234insT	p.Trp79Leufs X32	365-366insT	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	CFTR1 reference [3]: Claustres M, Altieri JP, Guittard C 2004-09-23 (reference not found on Pubmed).	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF in CFTR2.

c.3528delC	p.Lys1177SerfsX15	3659delC	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[6]	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.3717+12191C>T	No protein name	3849+10kbC->T	V	Splice	This variant causes CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	[16]	Class V splice mutation associated with reduced wild type CFTR function based on sequence analysis (intronic variant outside invariant splice site) and associated with PS-CF [2].
c.3717+5G>T	No protein name	3849+5G->T	Unclassified	Splice	Not described in CFTR2.	Not described in CFTR1 [3].	Splice mutation that is difficult to classify due to lack of data on residual CFTR function. This variant is outside the invariant splice site and likely associated with limited wild type CFTR function (class V), but this cannot be verified as the variant cDNA name, protein name and legacy name show no hits in Pubmed in the context of CF. The mutation is also not described in CFTR1 or CFTR2.
c.3773_3774insT	p.Leu1258PhefsX7	3905insT	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	CFTR1 reference [3]: Malik N, Hofmann S, Bosch-Al Jadooa N, Rutishauser M, Buhler E 1991-07-30 (reference not found on pubmed).	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.262_263delTT	p.Leu88IlefsX22	394delTT	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[17]	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF [2].

c.3884_3885insT	p.Ser1297PhefsX5	4016insT	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[18]	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affect a critical part of the protein and is associated with PI-CF [2].
c.4242+2T>C	No protein name	4374+2T->C	I	Splice	Not described in CFTR2.	CFTR1 reports 2 patients with suspected CF [3], unpublished.	Class I CFTR protein synthesis defect based on sequence analysis (a splice site mutation that affects an invariant splice site sequence (+1,+2, -1,-2), leading to inproper splicing of the intron-exon boundary). Although no disease classification is present in CFTR2, we classify invariant splice site sequence variations as class I defects due to the critical impact of invariant splice mutations on splicing. The variant cDNA name, protein name and legacy name show no hits in Pubmed in the context of CF.
c.4251delA	p.Glu1418ArgfsX14	4382delA	V	Ins/del	This variant causes CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	[19]	Class V mutation caused by a premature stop in the late C-terminus of the CFTR protein that is associated with residual CFTR function as evident by PS-CF status [2].
c.1210-33_1210-6GT[13]T[4]	No protein name	5T;TG13	V	Splice	This variant has varying consequences. Some patients with this variant has CF, when combined with another CF-causing variant. Other patients with this variant do not have CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	Not described in CFTR1 [3].	class V splice mutation based on sequence analysis (outside of the invariant splice sequence domain (+1,+2, -1,-2)) that affects expression level of wild type CFTR [20]. Associated with high residual CFTR function in intestinal organoids [21]. Varying clinical consequences are described in CFTR2 and following studies [22–24].
c.579+1G>T	No protein name	711+1G->T	I	Splice	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[25]	Class I CFTR protein synthesis defect based on sequence analysis (a splice site mutation that affects an invariant splice site sequence, leading to inproper splicing of the intron-exon boundary) and associated with PI-CF [2].

c.1364C>A	p.Ala455Glu	A455E	II	Missense	This variant causes CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	[6]	Class II trafficking defect associated with normal B band and low C band, but classification is complex based on multiple observations. A445E shows comparable single channel characteristics as wild type CFTR [26] but strongly reduced C-band upon expression in heterologous expression systems and in primary epithelial CF cells, yet more C band when compared to F508del [2, 27, 28]. A455E is also responsive to VX809 or other correctors, and to potentiators [28, 29]. Based on these data, we classified the primary defect as a class II processing defect.
c.137C>A	p.Ala46Asp	A46D	II	Missense	This variant causes CF when combined with another CF-causing variant. Insufficient data on pancreatic status.	[30]	Class II trafficking defect based on low C band in FRT cells and no response to ivacaftor [28].
c.(1584+1_1585-1)_(1679+1_1680-1)del	No protein name	CFTRdele11	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[31]	Class I CFTR protein synthesis defect based on DNA sequence analysis (large genetic deletion) associated with PI-CF in CFTR2.
c.(2988+1_2989-1)_(3367+1_3368-1)del	No protein name	CFTRdele17a,17b	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	Not described in CFTR1 [3].	Class I CFTR protein synthesis defect based on DNA sequence analysis (large genetic deletion) associated with PI-CF [2, 32].
No cDNA name	No protein name	CFTRdele19,20	I	Ins/del	Not described in CFTR2.	Not described in CFTR1 [3].	Class I CFTR protein synthesis defect based on DNA sequence analysis (large genetic deletion) associated with PI-CF [33].
c.54-5940_273+10250del21kb	p.Ser18ArgfsX16	CFTRdele2,3	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[34]	Class I CFTR protein synthesis defect based on DNA sequence analysis (large genetic deletion and downstream in frame PTC) associated with PI-CF [2].

c.3454G>C	p.Asp1152His	D1152H	IV	Missense	This variant has varying consequences. Some patients with this variant has CF, when combined with another CF-causing variant. Other patients with this variant do not have CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	[35]	Class IV mutation associated with altered pore function. D1152H is associated with a selective bicarbonate defective (CFTRBD) conductance [2, 36].
c.178G>T	p.Glu60X	E60X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	CFTR1 reference [3]: Malone G, Schwarz M, Super M 1991-11-22 (reference not available for full access).	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.2188G>T	p.Glu730X	E730X	I	Nonsense	Not described in CFTR2.	[37]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein.
c.274G>A	p.Glu92Lys	E92K	II	Missense	This variant causes CF when combined with another CF-causing variant. 44% (N=17) of patients in CFTR2 who have this variant are pancreatic insufficient.	[38]	Class II trafficking defect associated with strongly reduced CFTR maturation (C band) in heterologous cells systems [39, 40], and no response to ivacaftor [28]. Strong rescue by VX809 [2, 41].
c.1521_1523delCTT	p.Phe508del	F508del	II	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[42–44]	A class II trafficking defect associated with strongly reduced CFTR maturation (c band) in heterologous and primary cells systems which is considered dominant over additional defects that lower gating and surface retention[29, 39, 40, 45], associated with PI-CF [2].
c.3745G>A	p.Gly1249Arg	G1249R	Unclassified	Missense	This variant causes CF when combined with another CF-causing variant. 57% (N=4) of patients in CFTR2 who have this variant are pancreatic insufficient.	[46]	Organoids from G1249R/F508del showed prominent in vitro response to VX770 and not VX809, and clinical response to treatment with ivacaftor was observed [21]. It is likely a gating mutation based on ivacaftor sensitivity, but it might also have class IV defects. No papers could be found which experimentally characterize protein function. We therefore categorized this

							mutation as unclassified.
c.532G>A	p.Gly178Arg	G178R	III	Missense	This variant causes CF when combined with another CF-causing variant. 75% (N=57) of patients in CFTR2 who have this variant are pancreatic insufficient.	[25]	Ivacaftor responsive gating mutation associated with normal C band expression in heterologous expression models [47] and associated with PI-CF [2].
c.1381G>A	p.Gly461Arg	G461R	Unclassified	Missense	Not described in CFTR2.	Not described in CFTR1 [3].	F508del/G461R shows response to ivacaftor therapy both in vitro (organoids) and in vivo [48], however no papers have been published that experimentally characterize CFTR-protein. We therefore categorized this mutation as unclassified.
c.1624G>T	p.Gly542X	G542X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[6]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.1648G>T	p.Gly550X	G550X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	CFTR1 reference [3]: Deiman C, Deelan W, Halley D 1992-02-25 (reference not found on Pubmed)	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF.
c.1882G>A	p.Gly628Arg	G628R	II	Missense	This variant causes CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	[10]	Class II trafficking defect associated with strongly reduced C band expression in heterologous expression model and rescue by the corrector miglustat [49]. Based on these results we have classified this mutation as class II.
c.254G>A	p.Gly85Glu	G85E	II	Missense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[25]	Class II trafficking defect associated with strongly reduced C band expression in heterologous expression model and no response to ivacaftor [2, 28].

c.2908G>C	p.Gly970Arg	G970R	I	Splice	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[37]	Fidler et al. showed that the G>C change in the last position of the canonical 5′ splice donor site of exon 17 weakens the likelihood that this position will be recognized as a splice donor site and showed evidence that the G970R mutation must be reclassified primarily as a splice mutation [50], in contrast with previous work suggesting a gating defect [51].
c.3080T>C	p.Ile1027Thr	I1027T	Unclassified	Missense	This variant does not cause CF when combined with another CF-causing variant. There may patients in the CFTR2 database with this variant who have CF, but this variant is not the cause of their CF.	[10]	Non-CF causing polymorphism that was present here in cis with F508del which designated the complex allele as class II [2].
c.1007T>A	p.Ile336Lys	I336K	II	Missense	This variant causes CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	[37]	Class II trafficking defect associated with strongly reduced C band expression in heterologous expression model and no response to ivacaftor [2, 28].
c.1519_1521delATC	p.Ile507del	I507del	II	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[52]	Class II trafficking defect associated with strongly reduced C band expression in heterologous expression model [2, 53].
No cDNA name	No protein name	IVS11-1G->C	I	Splice	Not described in CFTR2.	Not described in CFTR1 [3].	Class I CFTR protein synthesis defect based on sequence analysis (a splice site mutation that affects an invariant splice site sequence, leading to inproper splicing of the intron-exon boundary).
No cDNA name	p.Leu1034Pro	L1034P	Unclassified	Missense	Not described in CFTR2.	Not described in CFTR1 [3].	Unclassified. Variant cDNA name, protein name and legacy name show no hits in Pubmed in the context of CF. The mutation is also not described in CFTR1 or CFTR2.
c.4004T>C	p.Leu1335Pro	L1335P	Unclassified	Missense	This variant causes CF when combined with another CF-causing variant. 61% (N=11) of patients in CFTR2 who have this variant are pancreatic insufficient.	CFTR1 reference [3]: Zielenski J, Tzountzouris J, Tsui L-C 1997-08-12 (reference not found on pubmed)	Unclassified. Mutation is listed as responsive to symdeko or trikafta (www.symdeko.com or www.trikafta.com).

c.617T>G	p.Leu206Trp	L206W	II	Missense	This variant causes CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	[19]	Class II trafficking defect associated with strongly reduced C band expression in heterologous expression model and no response to ivacaftor [2, 28].
c.2195T>G	p.Leu732X	L732X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	CFTR1 reference [3]: Malone G, Haworth A, Schwartz M 1994-10-05 (reference not available for full access)	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.2780T>C	p.Leu927Pro	L927P	Unclassified	Missense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[54]	Unclassified. L927P may cause cystic fibrosis by interfering with conformational changes necessary for channel opening [55]. The surface expression level of the L927P mutant is 43% that of the wild-type protein, but its channel activity is only 0.1% [28]. Others reported a normal protein expression [2].
c.3909C>G	p.Asn1303Lys	N1303K	II	Missense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[56]	Class II trafficking defect associated with strongly reduced C band expression in both heterologous and primary CF cells [29, 57]. It does not or hardly responds to ivacaftor or lumacaftor alone or in combination, but responds to VX-445 [2, 58].
c.4046delG	p.Gly1349AlafsX5	No legacy name	Unclassified	Ins/del	Not described in CFTR2.	Not described in CFTR1 [3].	CFTR protein synthesis defect, but due to the late position of the stopcodon it is unclear whether the resulting protein has some associated function.
c.4243-3T>A	No protein name	No legacy name	Unclassified	Splice	Not described in CFTR2.	CFTR1 reports obstructive lung function, bronciectasis and infections. Pancreatic sufficiency, no liver abnormalities, elevated sweat chloride level [3], but unpublished.	Variant cDNA name, protein name and legacy name show no hits in Pubmed in the context of CF. The mutation is also not described in CFTR2. It is unclear whether the splice defect can be classified as class I or V.
No cDNA name	p.Gln1012Pro	Q1012P	Unclassified	Missense	Not described in CFTR2.	Not described in CFTR1 [3].	Unclassified. Variant cDNA name, protein name and legacy name show no hits in Pubmed in the context of CF. The mutation is also not described in CFTR1 or CFTR2.

c.1477C>T	p.Gln493X	Q493X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[6]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.3196C>T	p.Arg1066Cys	R1066C	II	Missense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[10]	Class II trafficking defect associated with strongly reduced C band expression in heterologous cell expression systems and no response to ivacaftor [2, 28, 59].
c.3197G>A	p.Arg1066His	R1066H	II	Missense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[60]	Class II trafficking defect associated with strongly reduced C band expression in heterologous cell expression systems and no response to ivacaftor [2, 28].
c.3484C>T	p.Arg1162X	R1162X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[61]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.350G>A	p.Arg117His	R117H	IV	Missense	This variant has varying consequences. Some patients with this variant have CF, when combined with another CF-causing variant. Other patients with this variant do not have CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	[62]	R117H is associated with altered conductance properties and was originally classified as class IV [63]. It is a complex allele associated with a intronic polyT tract (5T, 7T or 9T) that affects splicing efficiency which associates with disease severity (class V). Others have also reported trafficking defects [64] or gating defects [65]. C-band expression in heterologous systems is mostly reflective of wt CFTR. R117H shows acute responsiveness to ivacaftor in heterologous systems and primary cells but only limited response to correctors [21]. For this study, we retain the original classification of R117H as class IV mutation [2].
c.4074A>T	p.Arg1358Ser	R1358S	Unclassified	Missense	Not described in CFTR2.	CFTR1 reference [3]: Férec C 1999-01-01 (reference not found on Pubmed).	Unclassified. Variant cDNA name, protein name and legacy name show no hits in Pubmed in the context of CF. The mutation is also not described in CFTR2.

c.1000C>T	p.Arg334Trp	R334W	IV	Missense	This variant causes CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	[66]	Normal CFTR protein expression in heterologous system but altered single channel conductance characteristic of class IV [63]. Some response to ivacaftor but not lumacaftor in primary CF cells [2, 21].
c.1040G>C	p.Arg347Pro	R347P	II	Missense	This variant causes CF when combined with another CF-causing variant. 64% (N=302) of patients in CFTR2 who have this variant are pancreatic insufficient.	[62]	Class II trafficking defect associated with reduced C band expression in heterologous cell expression systems. R347P was originally found to have altered channel conductance properties, but also matures very inefficiently (c-band ~15% of wild-type and is not associated with function despite some expression [28]. Moreover, (F508del/R347P) shows no clear detectable response to ivacaftor in primary CF cells [21]. Others also found processing defects [39]. This supports a primary defect in processing (class II). The limited product that reaches the surface has likely altered channel properties [2].
c.1657C>T	p.Arg553X	R553X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[67]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.221G>C	p.Arg74Pro	R74P	Unclassified	Missense	Not described in CFTR2.	Not described in CFTR1 [3].	Unclassified. Variant cDNA name, protein name and legacy name show no hits in Pubmed in the context of CF. The mutation is also not described in CFTR1 or CFTR2.
c.2290C>T	p.Arg764X	R764X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[15]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.2353C>T	p.Arg785X	R785X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[68]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF in CFTR2.

c.3752G>A	p.Ser1251Asn	S1251N	III	Missense	This variant causes CF when combined with another CF-causing variant. 72% (N=84) of patients in CFTR2 who have this variant are pancreatic insufficient.	[69]	Classified as ivacaftor responsive class III gating mutation with normal C-band expression [2, 47].
c.53G>T	p.Ser18Ile	S18I	Unclassified	Missense	Not described in CFTR2.	Not described in CFTR1 [3].	Unclassified. Variant cDNA name, protein name and legacy name show no hits in Pubmed in the context of CF. The mutation is also not described in CFTR1 or CFTR2.
c.1466C>A	p.Ser489X	S489X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	CFTR1 reference [3]: Macdonald K, Haworth, A Malone G, Schwarz M 1994-08-15 (reference not found on Pubmed)	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF [2, 70].
c.4186A>C	p.Thr1396Pro	T1396P	Unclassified	Missense	Not described in CFTR2.	Not described in CFTR1 [3].	Unclassified. One study reported no consistent manifestations of CF over time [71].
c.3477delT	p.Val1160X	V1160X	I	Nonsense	Not described in CFTR2.	[72]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein.
c.3846G>A	p.Trp1282X	W1282X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[73]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.2036G>A	p.Trp679X	W679X	I	Nonsense	Not described in CFTR2.	CFTR1 reference [3]: Walker C, Tsui L-C, Zielenski J 1999-09-27 (reference not found on Pubmed).	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein.
c.2537G>A	p.Trp846X	W846X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[74]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF [2].

c.3276C>A	p.Tyr1092X	Y1092X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[75]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.325T>G	p.Tyr109Asp	Y109D	Unclassified	Missense	Not described in CFTR2.	Not described in CFTR1 [3].	Unclassified. One report [76] described mutation as CF-causing, elevated sweat chloride levels and pancreatic insufficiency. No characterization of protein function has been published.
c.2547C>A	p.Tyr849X	Y849X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[77]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF in CFTR2.

Overview of the individual mutations in cDNA, protein and legacy name (according to the human genome variation society (HGVS) nomenclature) and corresponding CFTR classification including the rationale behind classification. The rationale is based on available literature and the clinical consequence of the mutation found in the CFTR2 database. In addition to the rationale, the original report describing the mutation for the first time was added to the table, derived from the CFTR1 database.

Supplementary table 2. Individual genotypes of study participants.

ID	Genotype (legacy name)	Genotype classification	Mutation group
1	G542X/CFTRdele2.3	Class I	ins/del-nonsense
2	1811+1G>C/1811+1G>C	Class I	splice-splice
3	1717-1G>A/2183AA>G	Class I	ins/del-splice
4	W1282X/W1282X	Class I	nonsense-nonsense
5	G542X/W679X	Class I	nonsense-nonsense
6	1811+1G>C/1811+1G>C	Class I	splice-splice
7	R785X/R785X	Class I	nonsense-nonsense
8	1717-1G>A/3905insT	Class I	ins/del-splice
9	R1162X/3659delC	Class I	ins/del-nonsense
10	1811+1G>C/1811+1G>C	Class I	splice-splice
11	711+1G>T/CFTRdele11	Class I	ins/del-splice
12	1677delTA/3120+1G>A	Class I	ins/del-splice
13	L732X/L732X	Class I	nonsense-nonsense
14	1811+1G>C/1811+1G>C	Class I	splice-splice
15	711+1G>T/711+1G>T	Class I	splice-splice
16	F508del/L206W	Class II	ins/del-missense
17	F508del/G628R	Class II	ins/del-missense
18	F508del/I336K	Class II	ins/del-missense
19	A455E/3659delC	Class II	ins/del-missense
20	A455E/1343delG	Class II	ins/del-missense
21	R1066H/CFTRdele2.3	Class II	ins/del-missense
22	F508del/G628R	Class II	ins/del-missense
23	A455E/E60X	Class II	missense-nonsense
24	F508del/G628R	Class II	ins/del-missense
25	F508del/F508del	Class II	ins/del-ins/del
26	G542X/R1066C	Class II	missense-nonsense
27	F508del/2184delA	Class II	ins/del-ins/del
28	F508del/R347P	Class II	ins/del-missense
29	F508del/Y1092X	Class II	ins/del-nonsense
30	F508del/365-366insT(W79fs)	Class II	ins/del-ins/del
31	F508del/R1066C	Class II	ins/del-missense
32	F508del/1342-1delG	Class II	ins/del-splice
33	F508del/W846X	Class II	ins/del-nonsense
34	F508del/1717-1G>A	Class II	ins/del-splice
35	F508del/CFTRdele17a.17b	Class II	ins/del-ins/del
36	F508del/F508del	Class II	ins/del-ins/del
37	R1066C/R1066H	Class II	missense-missense
38	V1160X/E92K	Class II	missense-nonsense

39	F508del/1078delT	Class II	ins/del-ins/del
40	F508del/W1282X	Class II	ins/del-nonsense
41	F508del/R347P	Class II	ins/del-missense
42	F508del/1078delT	Class II	ins/del-ins/del
43	F508del/F508del	Class II	ins/del-ins/del
44	F508del/F508del	Class II	ins/del-ins/del
45	N1303K/G550X	Class II	missense-nonsense
46	F508del/CFTRdele19.20	Class II	ins/del-ins/del
47	F508del/F508del	Class II	ins/del-ins/del
48	F508del/R347P	Class II	ins/del-missense
49	F508del/I336K	Class II	ins/del-ins/del
50	A46D/A46D	Class II	missense-missense
51	F508del/3659delC	Class II	ins/del-ins/del
52	F508del/CFTRdele17a.17b	Class II	ins/del-ins/del
53	F508del/E730X	Class II	ins/del-nonsense
54	F508del/2183AA>G	Class II	ins/del-ins/del
55	F508del/Y1092X	Class II	ins/del-nonsense
56	F508del/1813insC	Class II	ins/del-ins/del
57	F508del/E60X	Class II	ins/del-nonsense
58	F508del/711+1G>T	Class II	ins/del-splice
59	F508del/Y1092X	Class II	ins/del-nonsense
60	F508del/G85E	Class II	ins/del-missense
61	F508del/I507del	Class II	ins/del-ins/del
62	F508del/1717-1G>A	Class II	ins/del-splice
63	F508del/W1282X	Class II	ins/del-nonsense
64	F508del/3659delC	Class II	ins/del-ins/del
65	F508del/711+1G>T	Class II	ins/del-splice
66	F508del/1717-1G>A	Class II	ins/del-splice
67	F508del/CFTRdele17a.17b	Class II	ins/del-ins/del
68	F508del/Y849X	Class II	ins/del-nonsense
69	F508del/1717-1G>A	Class II	ins/del-splice
70	N1303K/G85E	Class II	missense-missense
71	F508del/CFTRdele2.3	Class II	ins/del-ins/del
72	F508del/CFTRdele17a.17b	Class II	ins/del-ins/del
73	A46D/A46D	Class II	missense-missense
74	F508del/Y849X	Class II	ins/del-nonsense
75	F508del/G85E	Class II	ins/del-missense
76	F508del/S489X	Class II	ins/del-nonsense
77	F508del/2184delA	Class II	ins/del-ins/del
78	G542X/R1066C	Class II	missense-nonsense
79	F508del/711+1G>T	Class II	ins/del-splice

80	F508del/F508del	Class II	ins/del-ins/del
81	F508del/W1282X	Class II	ins/del-nonsense
82	F508del/Y1092X	Class II	ins/del-nonsense
83	F508del/N1303K	Class II	ins/del-missense
84	F508del/711+1G>T	Class II	ins/del-splice
85	I507del/4374+2T->C	Class II	ins/del-splice
86	F508del/711+1G>T	Class II	ins/del-splice
87	F508del/R1162X	Class II	ins/del-nonsense
88	F508del/G550X	Class II	ins/del-nonsense
89	F508del/G550X	Class II	ins/del-nonsense
90	F508del/F508del	Class II	ins/del-ins/del
91	F508del/F508del	Class II	ins/del-ins/del
92	F508del/Q493X	Class II	ins/del-nonsense
93	F508del/4016insT	Class II	ins/del-ins/del
94	F508del/394delTT	Class II	ins/del-ins/del
95	F508del/IVS11-1G>C	Class II	ins/del-splice
96	F508del/G550X	Class II	ins/del-nonsense
97	F508del/R1162X	Class II	ins/del-nonsense
98	F508del/R1162X	Class II	ins/del-nonsense
99	F508del/Y1092X	Class II	ins/del-nonsense
100	F508del/N1303K	Class II	ins/del-missense
101	F508del/W1282X	Class II	ins/del-nonsense
102	F508del/2184insA	Class II	ins/del-ins/del
103	F508del/F508del	Class II	ins/del-ins/del
104	F508del/R1162X	Class II	ins/del-nonsense
105	F508del/E60X	Class II	ins/del-nonsense
106	F508del/R1162X	Class II	ins/del-nonsense
107	F508del/S1251N	Class III	ins/del-missense
108	F508del/S1251N	Class III	ins/del-missense
109	F508del/S1251N	Class III	ins/del-missense
110	F508del/S1251N	Class III	ins/del-missense
111	F508del/S1251N	Class III	ins/del-missense
112	F508del/S1251N	Class III	ins/del-missense
113	F508del/S1251N	Class III	ins/del-missense
114	F508del/S1251N	Class III	ins/del-missense
115	S1251N/1717-1G>A	Class III	missense-splice
116	F508del/S1251N	Class III	ins/del-missense
117	F508del/G178R	Class III	ins/del-missense
118	3905insT/D1152H	Class IV	ins/del-missense
119	F508del/D1152H	Class IV	ins/del-missense
120	W1282X/R117H;7T	Class IV	missense-nonsense

121	F508del/R117H;7T/9T	Class IV	ins/del-missense
122	R1162X/D1152H	Class IV	missense-nonsense
123	R117H;7T/R553X	Class IV	missense-nonsense
124	R334W/N1303K	Class IV	missense-missense
125	R334W/R334W	Class IV	missense-missense
126	D1152H/R1162X	Class IV	missense-nonsense
127	R334W/R764X	Class IV	missense-nonsense
128	F508del/5T;TG13	Class V	ins/del-splice
129	F508del/5T;TG13	Class V	ins/del-splice
130	G542X/3849+10kbC>T	Class V	nonsense-splice
131	F508del/5T;TG13	Class V	ins/del-splice
132	A455E/5T;TG13	Class V	missense-splice
133	F508del/3849+10kbC>T	Class V	ins/del-splice
134	F508del/3849+10kbC>T	Class V	ins/del-splice
135	F508del/3849+10kbC>T	Class V	ins/del-splice
136	3272-26A>G/3272-26A>G	Class V	splice-splice
137	F508del/3849+10kbC>T	Class V	ins/del-splice
138	3272-26A>G/G970R	Class V	splice-splice
139	F508del/3272-26A>G	Class V	ins/del-splice
140	F508del/3272-26A>G	Class V	ins/del-splice
141	F508del/3272-26A>G	Class V	ins/del-splice
142	3272-26A>G/1898+5G>T	Class V	splice-splice
143	F508del/3272-26A>G	Class V	ins/del-splice
144	4382delA/2043delG	Class V	ins/del-ins/del
145	F508del/4382delA	Class V	ins/del-ins/del
146	F508del/2789+5G>A	Class V	ins/del-splice
147	F508del/4382delA	Class V	ins/del-ins/del
148	Y849X/2789+5G>A	Class V	nonsense-splice
149	1078delT/3272-26A>G	Class V	ins/del-splice
150	3849+10kbC>T/1717-1G>A	Class V	splice-splice
151	F508del/c.4243-3T>A	Unclassified	ins/del-splice
152	F508del/R1358S	Unclassified	ins/del-missense
153	F508del;I1027T/UNK	Unclassified	ins/del-unknown
154	UNK/UNK	Unclassified	unknown-unknown
155	R553X/c.4243-3T>A	Unclassified	nonsense-splice
156	F508del/T1396P	Unclassified	ins/del-missense
157	F508del/G461R	Unclassified	ins/del-missense
158	N1303K/Q1012P	Unclassified	missense-missense
159	F508del/UNK	Unclassified	ins/del-unknown
160	R117H;7T/UNK	Unclassified	missense-unknown
161	F508del/G1249R	Unclassified	ins/del-missense

162	UNK/UNK	Unclassified	unknown-unknown
163	F508del/G1249R	Unclassified	ins/del-missense
164	F508del/UNK	Unclassified	ins/del- unknown
165	F508del/3849+5G>T	Unclassified	ins/del-splice
166	L1335P/L1335P	Unclassified	missense-missense
167	F508del/R74P	Unclassified	ins/del-missense
168	F508del/L1034P	Unclassified	ins/del-missense
169	F508del/S18I	Unclassified	ins/del-missense
170	F508del/Y109D	Unclassified	ins/del-missense
171	W1282X/L927P	Unclassified	missense-nonsense
172	F508del/UNK	Unclassified	ins/del-unknown
173	F508del/c.4046delG	Unclassified	ins/del-ins/del

Overview of individual genotypes with corresponding CFTR mutation classification according to the rationale described in supplementary table 1. Genotypes are provided in legacy name, unless stated otherwise (c. = cDNA code). Study participants were categorized into one mutation class based on the mildest of both mutation classes, or to unclassified when one of the mutation classes was unknown or uncertain. Mutation group was defined by the combination of mutation types of both alleles.

Supplementary table 3. Association of FIS with FEV1pp decline in subgroup analysis 4-25 years.

Regression coefficients of linear mixed effects model for FEV1pp within a subgroup including participants between 4-25 years of age.

N=107, obs=644	**Coefficient (95% CI)**	**P-value**
Age	-1.57 (-2.03 – -1.10)	<0.001*
FIS	-3.01 (-11.07 – 5.04)	0.462
FIS*age	0.49 (0.03 – 0.96)	0.039*
Treatment		
- none	Reference category	
- ivacaftor	9.63 (4.93 – 14.33)	<0.001*
- lumacaftor/ivacaftor	-4.32 (-10.70 – 2.06)	0.184
Sex		
- male	Reference category	
- female	0.16 (-6.38 – 6.71)	0.961
Genotype class		
- unclassified	Reference category	
- class I	0.93 (-14.29 – 16.16)	0.904
- class II	6.21 (-6.30 – 18.72)	0.330
- class III	7.86 (-6.99 – 22.71)	0.299
- class IV	21.37 (1.52 – 41.22)	0.349
- class V	-1.58 (-20.64 – 17.47)	0.870
SCC	-0.09 (-0.25 – 0.07)	0.264

*FEV1pp: Forced expiratory volume in 1 second, percent predicted. Age in years. FIS: Forskolin-induced swelling, defined as the relative size increase of intestinal organoids (AUC) after 1h stimulation with 0.8 µM/L forskolin, coefficient scaled 1:1000 AUC. SCC: Sweat chloride concentration in mmol/L. FIS*age indicates the difference in annual FEV1pp decline per 1000 AUC change in FIS level. Genotype class: CFTR protein function class of the mildest of both CFTR mutations. * Significance level P < 0.05.*

Supplementary table 4. Association of FIS with FEV1pp decline in sensitivity analysis including genotype group.

Regression coefficients of linear mixed effects model for FEV1pp with genotype group and subgroup only including participants between 4 – 25 years of age.

	N=138, obs=970		Subgroup: N=100, obs=601	
	Coefficient (95% CI)	**P-value**	**Coefficient (95% CI)**	**P-value**
Age	-1.25 (-1.54 – -0.96)	<0.001*	-1.68 (-2.15 – -1.21)	<0.001*
FIS	-2.93 (-8.42 – 2.56)	0.295	-5.22 (-12.15 – 1.71)	0.140
FIS*age	0.36 (0.12 – 0.61)	0.004*	0.69 (0.17 – 1.20)	0.009*
Treatment				
- none	Reference category		Reference category	
- ivacaftor	8.43 (4.79 – 12.06)	<0.001*	10.03 (4.88 – 15.17)	<0.001*
- lumacaftor/ivacaftor	-3.44 (-7.89 – 1.00)	0.129	-4.30 (-10.66 – 2.06)	0.185
Sex				
- male	Reference category		Reference category	
- female	-0.07 (-6.21 – 6.07)	0.982	0.82 (-5.72 – 7.36)	0.805
Genotype group				
- Ins/del – missense	Reference category		Reference category	
- Ins/del – nonsense	-0.24 (-9.98 – 9.51)	0.962	2.05 (-7.94 – 12.05)	0.687
- Ins/del – splice	-2.91 (-12.08 – 6.25)	0.533	-2.29 (-11.89 – 7.31)	0.640
- Ins/del – ins/del	-0.45 (-10.05 – 9.14)	0.927	1.56 (-8.72 – 11.84)	0.766
- Missense – nonsense	7.85 (-6.51 – 22.21)	0.284	8.40 (-8.39 – 25.19)	0.326
- Missense – missense	8.77 (-5.46 – 23.00)	0.227	13.84 (-1.12 – 28.79)	0.070
- Splice – splice	-6.30 (-21.46 – 8.87)	0.415	1.74 (-15.01 – 18.50)	0.838
SCC	-0.09 (-0.24 – 0.07)	0.289	-0.12 (-0.29 – 0.05)	0.176

*FEV1pp: Forced expiratory volume in 1 second, percent predicted. Age in years. FIS: Forskolin-induced swelling, defined as the relative size increase of intestinal organoids (AUC) after 1h stimulation with 0.8 µM/L forskolin, coefficient scaled 1:1000 AUC. FIS*age indicates the difference in annual FEV1pp decline per 1000 AUC change in FIS level. SCC: Sweat chloride concentration in mmol/L. Genotype group: combination of CFTR mutation types on both alleles. * Significance level P < 0.05.*

Supplementary table 5. Association of SCC with FEV1pp decline.

N=149, obs=1054	Coefficient (95% CI)	P-value
Age	--0.26 (-1.12 – 0.61)	0.563
FIS	1.78 (-4.05 – 7.62)	0.549
SCC	0.004 (-0.19 – 0.20)	0.971
SCC*age	-0.01 (-0.02 – 0.002)	0.121
Treatment		
- none	Reference category	
- ivacaftor	8.04 (4.61 – 11.47)	<0.001*
- lumacaftor/ivacaftor	-3.98 (-8.44 – 0.49)	0.081
Sex		
- male	Reference category	
- female	-0.75 (-6.83 – 5.32)	0.807
Genotype class		
- unclassified	Reference category	
- class I	0.73 (-13.51 – 14.97)	0.920
- class II	5.37 (-5.62 – 16.36)	0.338
- class III	10.66 (-3.45 – 24.76)	0.138
- class IV	12.11 (-4.46 – 28.69)	0.152
- class V	0.23 (-14.60 – 15.06)	0.976

*FEV1pp: Forced expiratory volume in 1 second, percent predicted. Age in years. FIS: Forskolin-induced swelling, defined as the relative size increase of intestinal organoids (AUC) after 1h stimulation with 0.8 µM/L forskolin, coefficient scaled 1:1000 AUC. SCC: Sweat chloride concentration in mmol/L. FIS*age indicates the difference in annual FEV1pp decline per 1000 AUC change in FIS level. Genotype class: CFTR protein function class of the mildest of both CFTR mutations. * Significance level P < 0.05.*

Supplementary table 6. Comparison of the association between FEV1pp decline and FIS versus SCC.

N=149, obs=1054	**Coefficient (95% CI)**	**P-value**
Age	-1.16 (-2.36 – 0.03)	0.056
FIS	-2.42 (-9.15 – 4.30)	0.480
FIS*age	0.33 (0.05 – 0.60)	0.020*
SCC	-0.09 (-0.31 – 0.12)	0.392
SCC*age	0.00 (-0.01 – 0.01)	0.995
Treatment		
- none	Reference category	
- ivacaftor	8.02 (4.61 – 11.43)	<0.001*
- lumacaftor/ivacaftor	-3.75 (-8.20 – 0.70)	0.098
Sex		
- male	Reference category	
- female	-0.99 (-7.06 – 5.07)	0.748
Genotype class		
- unclassified	Reference category	
- class I	0.51 (-13.65 – 14.67)	0.944
- class II	5.34 (-5.60 – 16.27)	0.339
- class III	10.25 (-3.82 – 24.32)	0.153
- class IV	11.09 (-5.44 – 27.63)	0.188
- class V	-2.45 (-17.33 – 12.43)	0.747

*FEV1pp: Forced expiratory volume in 1 second, percent predicted. Age in years. FIS: Forskolin-induced swelling, defined as the relative size increase of intestinal organoids (AUC) after 1h stimulation with 0.8 µM/L forskolin, coefficient scaled 1:1000 AUC. SCC: Sweat chloride concentration in mmol/L. FIS*age indicates the difference in annual FEV1pp decline per 1000 AUC change in FIS level. SCC*age indicates the difference in annual FEV1pp decline per 1-unit change in SCC level. Genotype class: CFTR protein function class of the mildest of both CFTR mutations. * Significance level P < 0.05.*

SUPPLEMENTARY FIGURE LEGENDS

Supplementary figure 1. Individual FIS responses.

a) waterfall plot of FIS responses stimulated with 0.8 µM forskolin for 1 hour of all study participants. b) waterfall plot of FIS responses at 0.8 µM forskolin per mutation group. Groups were defined by the combination of the mutation type of both mutations. Bars represent mean+SD of replicates, ranging from n=2 to n=7. The numbers on the x-axes represent the participant number and correspond to the numbers in figure 1b-c. Genotypes are specified in supplementary table 2.

Supplementary figure 2. Association of FIS with long-term FEV1pp decline in subgroup 4-25 years.

Predicted FEV1pp decline based on model coefficients in supplementary table 3, illustrating the association between different levels of residual CFTR function and long-term FEV1pp decline in the subgroup analysis. The analysis was performed with FIS as continuous variable, yet for illustrative purposes predicted FEV1pp decline is plotted by steps of 1000 AUC from 4 to 25 years, reflecting the age range of the subgroup. Average predicted annual FEV1pp decline per AUC level is specified on the right.

REFERENCES

1. Claustres M, Gerrard B, White MB, Desgeorges M, Kjellberg P, Rollin B, Dean M. A new mutation (1078delT) in exon 7 of the CFTR gene in a southern French adult with cystic fibrosis. *Genomics* 1992; 13: 907–908.

2. Sosnay PR, Siklosi KR, Van Goor F, Kaniecki K, Yu H, Sharma N, Ramalho AS, Amaral MD, Dorfman R, Zielenski J, Masica DL, Karchin R, Millen L, Thomas PJ, Patrinos GP, Corey M, Lewis MH, Rommens JM, Castellani C, Penland CM, Cutting GR. Defining the disease liability of variants in the cystic fibrosis transmembrane conductance regulator gene. *Nat. Genet.* [Internet] Nature Publishing Group; 2013; 45: 1160–1167Available from: http://dx.doi.org/10.1038/ng.2745.

3. CFTR1 database [Internet]. Available from: http://www.genet.sickkids.on.ca/.

4. Salinas DB, Sosnay PR, Azen C, Young S, Raraigh KS, Keens TG, Kharrazi M. Benign and deleterious cystic fibrosis transmembrane conductance regulator mutations identified by sequencing in positive cystic fibrosis newborn screen children from California. *PLoS One* 2016; 11: 1–14.

5. Baranov VS, Gorbunova VN, Ivaschenko TE, Shwed NY, Osinovskaya NS, Kascheeva TK, Lebedev VM, Mikhailov A V., Vakharlovsky VG, Kuznetzova T V. Five years' experience of prenatal diagnosis of cystic fibrosis in the former U.S.S.R. *Prenat. Diagn.* 1992; .

6. Kerem BS, Zielenski J, Markiewicz D, Bozon D, Gazit E, Yahav J, Kennedy D, Riordan JR, Collins FS, Kommens JM, Tsui LC. Identification of mutations in regions corresponding to the two putative nucleotide (ATP)-binding folds of the cystic fibrosis gene. *Proc. Natl. Acad. Sci. U. S. A.* 1990; 87: 8447–8451.

7. Petreska L, Koceva S, Efremov GD. A donor splice site mutation (1811 + 1G-->C) in intron 11 of the CFTR gene identified in a patient of Macedonian origin. *Hum. Mutat.* 1996; 7: 375.

8. Chillón M, Casals T, Giménez J, Nunes V, Estivill X. Analysis of the CFTR gene in the Spanish population: SSCP-screening for 60 known mutations and identification of four new mutations (Q30X, A120T, 1812-1 G-->A, and 3667del4). *Hum. Mutat.* 1994; 3: 223–230.

9. Zielenski J, Markiewicz D, Lin S-P, Huang F-Y, Yang-Feng TL, Tsui L-C. Skipping of exon 12 as a

consequence of a point mutation (1898 + 5G → T) in the cystic fibrosis transmembrane conductance regulator gene found in a consanguineous Chinese family. *Clin. Genet.* [Internet] 1995; 47: 125–132Available from: https://doi.org/10.1111/j.1399-0004.1995.tb03944.x.

10. Fanen P, Ghanem N, Vidaud M, Besmond C, Martin J, Costes B, Plassa F, Goossens M. Molecular characterization of cystic fibrosis: 16 Novel mutations identified by analysis of the whole cystic fibrosis conductance transmembrane regulator (CFTR) coding regions and splice site junctions. *Genomics* 1992; 13: 770–776.

11. Kilinc MO, Ninis VN, Tolun A, Estivill X, Casals T, Savov A, Dagli E, Karakoc F, Demirkol M, Huner G, Ozkinay F, Demir E, Seculi JL, Pena J, Bousono C, Ferrer-Calvete J, Calvo C, Glover G, Kremenski I. Genotype-phenotype correlation in three homozygotes and nine compound heterozygotes for the cystic fibrosis mutation 2183AA→G shows a severe phenotype. *J. Med. Genet.* 2000; 37: 307–309.

12. Chevalier-Porst F, Bonardot AM, Gilly R, Chazalette JP, Mathieu M, Bozon D. Mutation analysis in 600 French cystic fibrosis patients. *J. Med. Genet.* 1994; 31: 541–544.

13. Prontera P, Isidori I, Mencarini V, Pennoni G, Mencarelli A, Stangoni G, Di Cara G, Verrotti A. A Clinical and Molecular Survey of 62 Cystic Fibrosis Patients from Umbria (Central Italy) Disclosing a High Frequency (2.4%) of the 2184insA Allele: Implications for Screening. *Public Health Genomics* 2017; 19: 336–341.

14. Highsmith WE, Burch LH, Zhou Z, Olsen JC, Strong T V, Smith T, Friedman KJ, Silverman LM, Boucher RC, Collins FS, Knowles MR. Identification of a splice site mutation (2789 +5 G > A) associated with small amounts of normal CFTR mRNA and mild cystic fibrosis. *Hum. Mutat.* 1997; 9: 332–338.

15. Macek M, Mackova A, Hamosh A, Hilman BC, Selden RF, Lucotte G, Friedman KJ, Knowles MR, Rosenstein BJ, Cutting GR. Identification of common cystic fibrosis mutations in African-Americans with cystic fibrosis increases the detection rate to 75%. *Am. J. Hum. Genet.* 1997; 60: 1122–1127.

16. Highsmith WE, Burch LH, Zhou Z, Olsen JC, Boat TE, Spock A, Gorvoy JD, Quittel L, Friedman KJ, Silverman LM. A novel mutation in the cystic fibrosis gene in patients with pulmonary disease but normal sweat chloride concentrations. *N. Engl. J. Med.* 1994; 331: 974–980.

17. Schwartz M, Anvret M, Claustres M, Eiken HG, Eiklid K, Schaedel C, Stolpe L, Tranebjaerg L. 394delTT: a Nordic cystic fibrosis mutation. *Hum. Genet.* 1994; 93: 157–161.

18. Cheadle JP, Al-Jader LN, Meredith AL. Two novel frame-shift mutations: 977 insA in exon 6B, and 4016 insT in exon 21, of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. *Hum. Mol. Genet.* [Internet] 1993; 2: 317–319Available from: https://doi.org/10.1093/hmg/2.3.317.

19. Claustres M, Laussel M, Desgeorges M, Giansily M, Culard J-F, Razakatsara G, Demaille J. Analysis of the 27 exons and flanking regions of the cystic fibrosis gene: 40 different mutations account for 91.2% of the mutant alleles in Southern France. *Hum. Mol. Genet.* [Internet] 1993; 2: 1209–1213Available from: https://doi.org/10.1093/hmg/2.8.1209.

20. Nissim-Rafinia M, Chiba-Falek O, Sharon G, Boss A, Kerem B. Cellular and viral splicing factors can modify the splicing pattern of CFTR transcripts carrying splicing mutations. *Hum. Mol. Genet.* 2000; 9: 1771–1778.

21. Dekkers JF, Berkers G, Kruisselbrink E, Vonk A, De Jonge HR, Janssens HM, Bronsveld I, Van De Graaf EA, Nieuwenhuis EES, Houwen RHJ, Vleggaar FP, Escher JC, De Rijke YB, Majoor CJ, Heijerman HGM, De Winter – De Groot KM, Clevers H, Van Der Ent CK, Beekman JM. Characterizing responses to CFTR-modulating drugs using rectal organoids derived from subjects with cystic fibrosis. *Sci. Transl. Med* 2016; 8: 344–384.

22. Kerem E, Rave-Harel N, Augarten A, Madgar I, Nissim-Rafinia M, Yahav Y, Goshen R, Bentur L, Rivlin J, Aviram M, Genem A, Chiba-Falek O, Kraemer MR, Simon A, Branski D, Kerem B. A cystic fibrosis transmembrane conductance regulator splice variant with partial penetrance associated with variable cystic fibrosis presentations. *Am. J. Respir. Crit. Care Med.* 1997; 155: 1914–1920.

23. Hefferon TW, Groman JD, Yurk CE, Cutting GR. A variable dinuclelotide repeat in the CFTR gene contributes to phenotype diversity by forming RNA secondary structures that after splicing. *Proc. Natl. Acad. Sci. U. S. A.* 2004; 101: 3504–3509.

24. Groman JD, Hefferon TW, Casals T, Bassas L, Estivill X, Des Georges M, Guittard C, Koudova M, Fallin MD, Nemeth K, Fekete G, Kadasi L, Friedman K, Schwarz M, Bombieri C, Pignatti PF, Kanavakis E, Tzetis

M, Schwartz M, Novelli G, D'Apice MR, Sobczynska-Tomaszewska A, Bal J, Stuhrmann M, Macek M, Claustres M, Cutting GR. Variation in a Repeat Sequence Determines Whether a Common Variant of the Cystic Fibrosis Transmembrane Conductance Regulator Gene Is Pathogenic or Benign. *Am. J. Hum. Genet.* 2004; 74: 176–179.

25. Zielenski J, Bozon D, Kerem, Markiewicz BD, Durie P, Rommens JM, Tsui LC. Identification of mutations in exons 1 through 8 of the cystic fibrosis transmembrane conductance regulator (CFTR) gene [Internet]. Genomics 1991.Available from: https://doi.org/10.1016/0888-7543(91)90504-8.

26. Sheppard DN, Ostedgaard LS, Winter MC, Welsh MJ. Mechanism of dysfunction of two nucleotide binding domain mutations in cystic fibrosis transmembrane conductance regulator that are associated with pancreatic sufficiency. *EMBO J.* 1995; 14: 876–883.

27. Cebotaru L, Rapino D, Cebotaru V, Guggino WB. Correcting the cystic fibrosis disease mutant, A455E CFTR. *PLoS One* 2014; 9: 3–8.

28. Van Goor F, Yu H, Burton B, Hoffman BJ. Effect of ivacaftor on CFTR forms with missense mutations associated with defects in protein processing or function. *J. Cyst. Fibros.* [Internet] European Cystic Fibrosis Society.; 2014; 13: 29–36Available from: http://dx.doi.org/10.1016/j.jcf.2013.06.008.

29. Dekkers JF, Gondra RAG, Kruisselbrink E, Vonk AM, Janssens HM, De Winter-De Groot KM, Van Der Ent CK, Beekman JM. Optimal correction of distinct CFTR folding mutants in rectal cystic fibrosis organoids. *Eur. Respir. J.* [Internet] 2016; 48: 451–458Available from: http://dx.doi.org/10.1183/13993003.01192-2015.

30. Tzetis M, Kanavakis E, Antoniadi T, Traeger-Synodinos J, Doudounakis S, Adam G, Kattamis C. Identification of two novel mutations (296 + 1G - C and A46D) in exon 2 of the CFTR gene in Greek cystic fibrosis patients. *Mol. Cell. Probes* 1995; 9: 283–285.

31. Audrézet M-P, Chen J-M, Raguénès O, Chuzhanova N, Giteau K, Maréchal C Le, Quéré I, Cooper DN, Férec C. Genomic rearrangements in the CFTR gene: extensive allelic heterogeneity and diverse mutational mechanisms. *Hum. Mutat.* 2004; 23: 343–357.

32. Niel F, Martin J, Dastot-Le Moal F, Costes B, Boissier B, Delattre V, Goossens M, Girodon E. Rapid

detection of CFTR gene rearrangements impacts on genetic counselling in cystic fibrosis. *J. Med. Genet.* 2004; 41: 1–7.

33. Costes B, Girodon E, Vidaud D, Flori E, Ardalan A, Conteville P, Fanen P, Niel F, Vidaud M, Goossens M. Prenatal detection by real-time quantitative PCR and characterization of a new CFTR deletion, 3600+15kbdel5.3kb (or CFTRdele19). *Clin. Chem.* 2000; 46: 1417–1420.

34. Dörk T, Macek M, Mekus F, Tümmler B, Tzountzouris J, Casals T, Krebsová A, Koudová M, Sakmaryová I, Vávrová V, Zemková D, Ginter E, Petrova N V., Ivaschenko T, Baranov V, Witt M, Pogorzelski A, Bal J, Zékanowsky C, Wagner K, Stuhrmann M, Bauer I, Seydewitz HH, Neumann T, Jakubiczka S, Kraus C, Thamm B, Nechiporenko M, Livshits L, Mosse N, et al. Characterization of a novel 21-kb deletion, CFTRdele2,3(21 kb), in the CFTR gene: A cystic fibrosis mutation of Slavic origin common in Central and East Europe. *Hum. Genet.* 2000; 106: 259–268.

35. Jr WEH, Friedman KJ, Burch LH, Spock A, Silverman LM, Boucher RC, Knowles MR. A CFTR mutation (D1152H) in a family with mild lung disease and normal sweat chlorides. *Hum. Genet.* 2005; 68: 88–90.

36. LaRusch J, Jung J, General IJ, Lewis MD, Park HW, Brand RE, Gelrud A, Anderson MA, Banks PA, Conwell D, Lawrence C, Romagnuolo J, Baillie J, Alkaade S, Cote G, Gardner TB, Amann ST, Slivka A, Sandhu B, Aloe A, Kienholz ML, Yadav D, Barmada MM, Bahar I, Lee MG, Whitcomb DC. Mechanisms of CFTR Functional Variants That Impair Regulated Bicarbonate Permeation and Increase Risk for Pancreatitis but Not for Cystic Fibrosis. *PLoS Genet.* 2014; 10.

37. Cuppens H, Marynen P, De Boeck C, Cassiman JJ. Detection of 98.5% of the mutations in 200 Belgian cystic fibrosis alleles by reverse dot-blot and sequencing of the complete coding region and exon/intron junctions of the CFTR gene. *Genomics* 1993; 18: 693–697.

38. Nunes V, Chillón M, Dörk T, Tümmler B, Casals T, Estivill X. A new missense mutation (E92K) in the first transmembrane domain of the CFTR gene causes a benign cystic fibrosis phenotypeTitle. *Hum. Mol. Genet.* [Internet] 1993; 2: 79–80Available from: https://doi.org/10.1093/hmg/2.1.79.

39. Veit G, Avramescu RG, Chiang AN, Houck SA, Cai Z, Peters KW, Hong JS, Pollard HB, Guggino WB, Balch WE, Skach WR, Cutting GR, Frizzell RA, Sheppard DN, Cyr DM, Sorscher EJ, Brodsky JL, Lukacs GL. From

CFTR biology toward combinatorial pharmacotherapy: Expanded classification of cystic fibrosis mutations. *Mol. Biol. Cell* 2016; 27: 424–433.

40. Ensinck M, De Keersmaecker L, Heylen L, Ramalho AS, Gijsbers R, Farré R, De Boeck K, Christ F, Debyser Z, Carlon MS. Phenotyping of Rare CFTR Mutations Reveals Distinct Trafficking and Functional Defects. *Cells* 2020; 9: 1–14.

41. Ren HY, Grove DE, De La Rosa O, Houck SA, Sopha P, Van Goor F, Hoffman BJ, Cyr DM. VX-809 corrects folding defects in cystic fibrosis transmembrane conductance regulator protein through action on membrane-spanning domain 1. *Mol. Biol. Cell* 2013; 24: 3016–3024.

42. Rommens J, Iannuzzi M, Kerem B, Drumm M, Melmer G, Dean M, Rozmahel R, Cole J, Kennedy D, Hidaka N, ZSIGA M, BUCHWALD M, TSUI L-C, RIORDAN JR, COLLINS FS. Identification of the Cystic Fibrosis Gene: Chromosome Walking and Jumping. *Science (80-.).* 1989; 245: 1059–1065.

43. Riordan JR, Rommens JM, Kerem B-S, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou J-L, Drumm ML, Iannuzzi MC, Collins FS, Tsui L-C. Identification of the Cystic Fibrosis Gene: Cloning and Characterization of Complementary DNA. *Science (80-.).* 1989; 245: 1066–1073.

44. Kerem B-S, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, Buchwald M, Tsui L-C. Identification of the Cystic Fibrosis Gene: Genetic Analysis. *Science (80-.).* 1989; 245: 1073–1080.

45. Cheng SH, Gregory RJ, Marshall J, Paul S, Souza DW, White GA, O'Riordan CR, Smith AE. Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. *Cell* 1990; 63: 827–834.

46. Dijkstra, Dirk-Jan W. Scheffer H, Buys CHCM. A novel mutation (G1249R) in exon 20 of the CFTR gene. *Hum. Mutat.* 1994; 4: 161–162.

47. Yu H, Burton B, Huang CJ, Worley J, Cao D, Johnson JP, Urrutia A, Joubran J, Seepersaud S, Sussky K, Hoffman BJ, Van Goor F. Ivacaftor potentiation of multiple CFTR channels with gating mutations. *J. Cyst. Fibros.* 2012; 11: 237–245.

48. Berkers G, van Mourik P, Vonk AM, Kruisselbrink E, Dekkers JF, de Winter-de Groot KM, Arets HGM, Marck-van der Wilt REP, Dijkema JS, Vanderschuren MM, Houwen RHJ, Heijerman HGM, van de Graaf

EA, Elias SG, Majoor CJ, Koppelman GH, Roukema J, Bakker M, Janssens HM, van der Meer R, Vries RGJ, Clevers HC, de Jonge HR, Beekman JM, van der Ent CK. Rectal Organoids Enable Personalized Treatment of Cystic Fibrosis. *Cell Rep.* [Internet] ElsevierCompany.; 2019; 26: 1701-1708.e3Available from: https://doi.org/10.1016/j.celrep.2019.01.068.

49. Billet A, Melin P, Jollivet M, Mornon JP, Callebaut I, Becq F. C terminus of nucleotide binding domain 1 contains critical features for cystic fibrosis transmembrane conductance regulator trafficking and activation. *J. Biol. Chem.* 2010; 285: 22132–22140.

50. Fidler MC, Buckley A, Sullivan JC, Statia M, Boj SF, Vries RGJ, Munck A, Higgins M, Moretto Zita M, Negulescu P, van Goor F, De Boeck K. G970R-CFTR Mutation (c.2908G>C) Results Predominantly in a Splicing Defect. *Clin. Transl. Sci.* 2021; 14: 656–663.

51. Seibert FS, Linsdell P, Loo TW, Hanrahan JW, Riordan JR, Clarke DM. Cytoplasmic loop three of cystic fibrosis transmembrane conductance regulator contributes to regulation of chloride channel activity. *J. Biol. Chem.* [Internet] Â© 1996 ASBMB. Currently published by Elsevier Inc; originally published by American Society for Biochemistry and Molecular Biology.; 1996; 271: 27493–27499Available from: http://dx.doi.org/10.1074/jbc.271.44.27493.

52. Schwarz M, Summers C, Heptinstall L, Newton C, Markham A, Super M. A deletion mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) locus: Delta I507. 1991; 290: 393–398.

53. Lopes-Pacheco M, Sabirzhanovaa I, Rapinoa D, Morales MM, Gugginoa WB, Cebotaru L. Correctors Rescue CFTR Mutations in Nucleotide-Binding Domain 1 (NBD1) by Modulating Proteostasis. *chembiochem* 2016; 17: 493–505.

54. Hermans CJ, Veeze HJ, Drexhage VR, Halley DJJ, van den Ouweland AMW. Identification of the L927P and ΔL1260 mutations in the CFTR gene. *Hum. Mol. Genet.* [Internet] 1994; 3: 1199–1200Available from: https://doi.org/10.1093/hmg/3.7.1199.

55. Zhang Z, Liu F, Chen J. Molecular structure of the ATP-bound, phosphorylated human CFTR. *Proc. Natl. Acad. Sci. U. S. A.* 2018; 115: 12757–12762.

56. Osborne L, Santis G, Schwarz M, Klinger K, Dörk T, McIntosh I, Schwartz M, Nunes V, Jr MM, Reiss J.

Incidence and expression of the N1303K mutation of the cystic fibrosis (CFTR) gene. *Hum. Genet.* 1992; 89: 653–658.

57. Gregory RJ, Rich DP, Cheng SH, Souza DW, Paul S, Manavalan P, Anderson MP, Welsh MJ, Smith AE. Maturation and function of cystic fibrosis transmembrane conductance regulator variants bearing mutations in putative nucleotide-binding domains 1 and 2. *Mol. Cell. Biol.* 1991; 11: 3886–3893.

58. Veit G, Roldan A, Hancock MA, da Fonte DF, Xu H, Hussein M, Frenkiel S, Matouk E, Velkov T, Lukacs GL. Allosteric folding correction of F508del and rare CFTR mutants by elexacaftor-tezacaftor-ivacaftor (Trikafta) combination. *JCI Insight* 2020; 5: 1–14.

59. Fanen P, Labarthe R, Garnier F, Benharouga M, Goossens M, Edelman A. Cystic fibrosis phenotype associated with pancreatic insufficiency does not always reflect the cAMP-dependent chloride conductive pathway defect. Analysis of C225R-CFTR and R1066C-CFTR. *J. Biol. Chem.* 1997; 272: 30563–30566.

60. Ferec C, Audrezet MP, Mercier B, Guillermit H, Moullier P, Quere I, Verlingue C. Detection of over 98% cystic fibrosis mutations in a Celtic population. 1992; 1: 188–191.

61. Nunes V, Gasparini P, Novelli G, Gaona A, Bonizzato A, Sangiuolo F, Balassopoulou A, Giménez FJ, Dognini M, Ravnik-Glavac M. Analysis of 14 cystic fibrosis mutations in five south European populations. *Hum. Genet.* 1991; 87: 737–738.

62. Dean M, White MB, Amos J, Gerrard B, Stewart C, Khaw KT, Leppert M. Multiple mutations in highly conserved residues are found in mildly affected cystic fibrosis patients. *Cell* 1990; 61: 863–870.

63. DN S, DP R, LS O, RJ G, AE S, MJ W. Mutations in CFTR associated with mild-disease-form Cl- channels with altered pore properties. *Nature* [Internet] 1993; 362: 160–164Available from: http://queens.ezp1.qub.ac.uk/login?url=http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=med3&AN=7680769%0Ahttp://resolver.ebscohost.com/openurl?sid=OVID:medline&id=pmid:7680769&id=doi:&issn=0028-0836&isbn=&volume=362&issue=6416&spage=1.

64. Gentzsch M, Ren HY, Houck SA, Quinney NL, Cholon DM, Sopha P, Chaudhry IG, Das J, Dokholyan N V., Randell SH, Cyr DM. Restoration of R117H CFTR folding and function in human airway cells through

combination treatment with VX-809 and VX-770. *Am J Physiol Lung Cell Mol Physiol.* 2016; 311: L550–L559.

65. Yu YC, Sohma Y, Hwang TC. On the mechanism of gating defects caused by the R117H mutation in cystic fibrosis transmembrane conductance regulator. *J. Physiol.* 2016; 594: 3227–3244.

66. Estivill X, Ortigosa L, Pérez-Frias J, J Dapena JF, Peña L, Peña L, Llevadot R, Giménez J, Nunes V. Clinical characteristics of 16 cystic fibrosis patients with the missense mutation R334W, a pancreatic insufficiency mutation with variable age of onset and interfamilial clinical differences. *Hum. Genet.* 1995; 95: 331–336.

67. Cutting GR, Kasch LM, Rosensteint BJ, Zielenskit J, Tsuit L, Antonarakis SE. A cluster of cystic fibrosis mutations in the first nucleotide-binding fold of the cystic fibrosis conductance regulator. 1990; 346: 366–369.

68. Hughes D, Wallace A, Taylor J, Tassabehji M, McMahon R, Hill A, Nevin N, Graham C. Fluorescent multiplex microsatellites used to define haplotypes associated with 75 CFTR mutations from the UK on 437 CF chromosomes. *Hum. Mutat.* 1996; 8: 229–235.

69. Kälin N, Dörk T, Tümmler B. A cystic fibrosis allele encoding missense mutations in both nucleotide binding folds of the cystic fibrosis transmembrane conductance regulator. *Hum. Mutat.* 1992; 1: 204–210.

70. De Bie I, Agatep R, Scott P, Ruchon A. Report on the p.Ser489X (p.Ser489) CFTR mutation, a variant with severe associated phenotype and high prevalence in a Quebec French-Canadian cystic fibrosis patient population. *Genet. Med.* 2012; 14: 883–886.

71. Prach L, Koepke R, Kharrazi M, Keiles S, Salinas DB, Reyes MC, Pian M, Opsimos H, Otsuka KN, Hardy KA, Milla CE, Zirbes JM, Chipps B, O'Bra S, Saeed MM, Sudhakar R, Lehto S, Nielson D, Shay GF, Seastrand M, Jhawar S, Nickerson B, Landon C, Thompson A, Nussbaum E, Chin T, Wojtczak H. Novel CFTR variants identified during the first 3 years of cystic fibrosis newborn screening in California. *J. Mol. Diagnostics* [Internet] American Society for Investigative Pathology; 2013; 15: 710–722Available from: http://dx.doi.org/10.1016/j.jmoldx.2013.05.006.

72. Dayangaç-Erden D, Atalay M, Emiralioğlu N, Hızal M, Polat S, Özçelik U, Yalçın E, Doğru D, Yılmaz E, Kiper N. Mutations of the CFTR gene and novel variants in Turkish patients with cystic fibrosis: 24-years experience. *Clin. Chim. Acta* 2020; 510: 252–259.

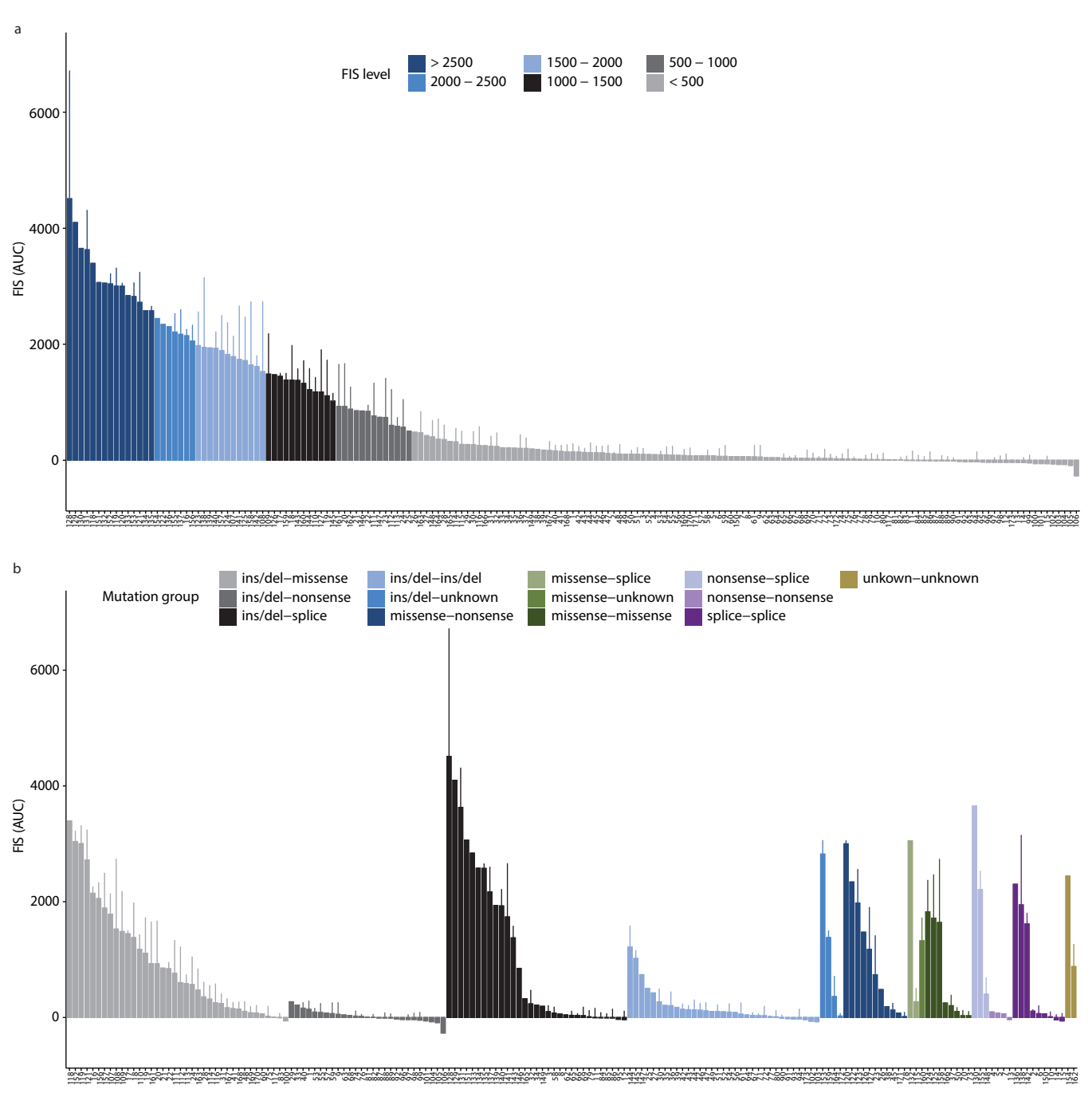
73. Vidaud M, Fanen P, Martin J, Ghanem N, Nicolas S, Goossens M. Three point mutations in the CFTR gene in French cystic fibrosis patients: identification by denaturing gradient gel electrophoresis. *Hum. Genet.* 1990; 85: 446–449.

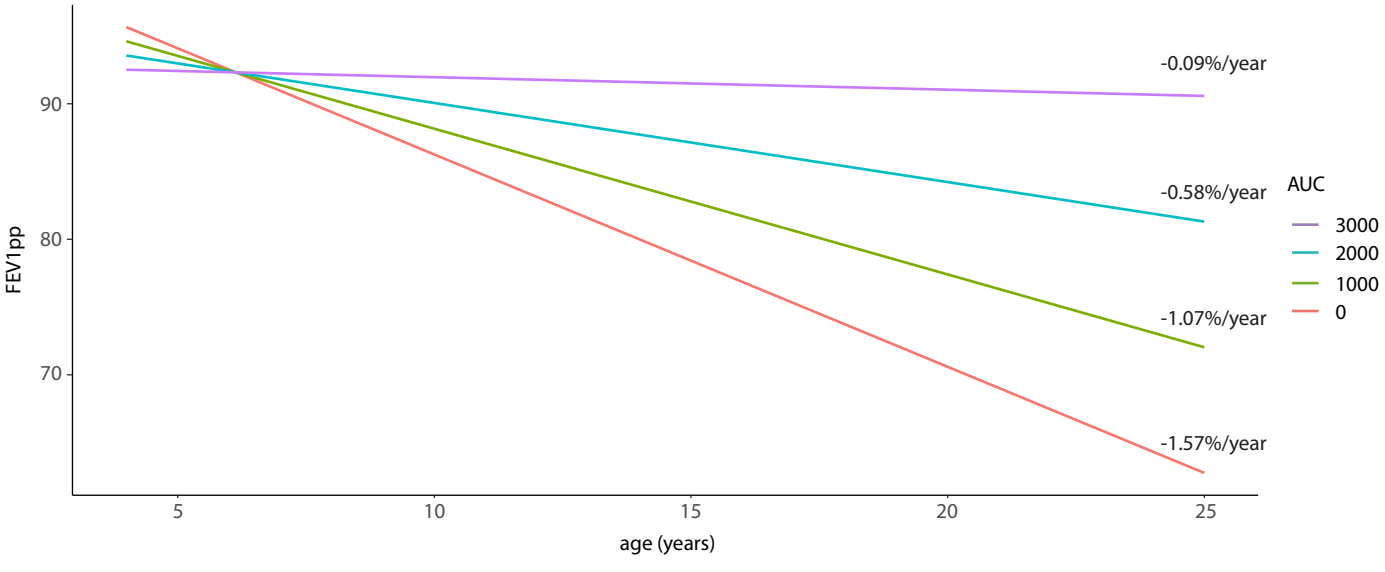
74. Cheadle JP, Al-Jader LN, Meredith AL. A novel nonsense mutation, W846XI (amber termination), in exon 14a of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. *Hum. Mol. Genet.* [Internet] 1993; 2: 1067–1068Available from: https://doi.org/10.1093/hmg/2.7.1067.

75. Bozon D, Zielenski J, Rininsland F, Lap-Chee Tsui. Identification of four new mutations in the cystic fibrosis transmembrane conductance regulator gene: I148T, L1077P, Y1092X, 2183AA→G. *Hum. Mutat.* [Internet] 1994; 3: 330–332Available from: https://doi.org/10.1002/humu.1380030329.

76. Liu K, Xu W, Xiao M, Zhao X, Bian C, Zhang Q, Song J, Chen K, Tian X, Liu Y, Xu KF, Zhang X. Characterization of clinical and genetic spectrum of Chinese patients with cystic fibrosis. *Orphanet J. Rare Dis.* Orphanet Journal of Rare Diseases; 2020; 15: 1–10.

77. Castaldo G, Fuccio A, Cazeneuve C, Picci L, Salvatore D, Scarpa M, Goossens M, Salvatore F. A noval nonsense mutation (Y849X) in the CFTR gene of a CF patient from southern Italy. *Hum. Mutat.* 1999; 14: 272.


a
FIS level
> 2500
2000 – 2500
1500 – 2000
1000 – 1500
500 – 1000
< 500
FIS (AUC)
6000
4000
2000
0
b
Mutation group
ins/del–missense
ins/del–nonsense
ins/del–splice
ins/del–ins/del
ins/del–unknown
missense–nonsense
missense–splice
missense–unknown
missense–missense
nonsense–splice
nonsense–nonsense
splice–splice
unkown–unknown
FIS (AUC)
6000
4000
2000
0


-0.09%/year
-0.58%/year
-1.07%/year
-1.57%/year
AUC
3000
2000
1000
0
FEV1pp
90
80
70
5
10
15
20
25
age (years)