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Factors associated with FEV1 decline in cystic fibrosis: analysis of the data of the ECFS Patient Registry

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Abstract

Background

Pulmonary insufficiency is the main cause of death in cystic fibrosis (CF). The European CF Society Patient Registry (ECFSPR) offers a unique database to evaluate potential risk factors of CF lung disease in a heterogeneous population.

Methods

We analysed forced expiratory volume in one second (FEV1%) data of 14732 patients registered in the ECFSPR in year 2007. Linear and logistic regressions were used to investigate association between FEV1% and age, age at diagnosis, sex, pancreatic status, chronic *P. aeruginosa* infection, CF-related diabetes (CFRD), BMI, and genotype. Estimates were adjusted for country.

Findings

BMI, chronic infection by *P. aeruginosa*, pancreatic status and CFRD showed a statistically significant (all $p<0{\cdot}0001$) and clinically relevant effect on FEV1%. Age at diagnosis, sex and genotype had a statistically significant ($p<0{\cdot}01$), but not clinically relevant effect. We estimate that patients with lower BMI experience a 6 fold increased odds (95% CI 5·0;7.3) of having severe lung disease (FEV1<40%) compared to patients with normal BMI, and that patients affected by CFRD have a 1·8 fold increased odds (1·6;2·2) compared to patients not affected. Being chronically infected with *P. aeruginosa* increases the odds of severe lung disease by 2·4 (2·0;2·7) and pancreatic insufficient patients experience a 2·0 fold increased odds (1·6;2·5) of severe lung disease compared to pancreatic sufficient patients.

Interpretation

Nutrition, chronic *P. aeruginosa* infection, pancreatic status and CFRD were found to be potential risk factors of pulmonary function in patients with CF regardless of age. Since three of these factors are preventable or treatable, we emphasize the importance of their early identification through frequent routine tests, the implementation of infection control measures, and a timely initiation of antibiotic therapies. Furthermore, epidemiological and clinical studies comparing CF patients from different populations should control for these factors to avoid confounding.

Introduction

Despite considerable improvement in median survival, cystic fibrosis (CF) remains a life-shortening disease with pulmonary insufficiency as the main cause of death. Several methods are used to assess pulmonary disease severity in CF, including spirometry, chest imaging, and quality of life questionnaires. For prognosis, forced expiratory volume in 1 second (FEV1) compared to a reference population (FEV1 in % of predicted), is regarded as the best generally available measure for assessing CF lung disease[1]. FEV1 is currently still an influential driver for the definition of disease stage, for decisions on treatment[1,2], for comparison between centres and countries[3–5], as a primary outcome in clinical studies[6,7], and in the regulatory approval of CF respiratory therapies[8].
The European Cystic Fibrosis Society Patient Registry (ECFSPR) collects data on an annual basis according to agreed definitions and formats of a common set of variables[9] from individual CF centres and national CF registries in Europe and the neighbouring countries. Data collection in 2007 included over 20000 CF patients from 16 countries[10]. Such data collection is a reflection of the reality of CF across Europe, offering a unique basis for epidemiological analyses due to its international nature: it offers the possibility to evaluate factors potentially associated with FEV1 in a population that shows a wide variability of both lung disease and potential risk factors.
The aim of this study was to inspect the association between FEV1 and factors previously described in the literature as major risk factors of CF lung disease.

Patients and methods

We used data from patients with CF registered in the ECFSPR in the year 2007. Data were provided by the national CF registries of Belgium, Czech Republic, Denmark, France, Germany, Israel, The Netherlands, and United Kingdom and by individual CF centres from Austria, Bulgaria, Greece, Italy, Portugal, and Slovenia.
We analysed the association of the following variables with FEV1: age at diagnosis, age at FEV1 measurement, sex, pancreatic status, chronic *Pseudomonas aeruginosa* infection, CF-Related Diabetes (CFRD), BMI, and genotype. These variables had a satisfactory level of completeness in the ECFSPR database (Table1).
In this study, pancreatic insufficiency (PI) was defined as the use of enzymes and pancreatic sufficiency (PS) as no use of enzymes during 2007. Chronic infection by *P. aeruginosa* was defined according to the modified Leeds criteria and/or presence of antibodies[9] by the clinicians at each centre. United Kingdom defined chronic infection when patients have 3 or more positive isolates during the last 12 months. CFRD was identified as use of insulin during 2007. We investigated the effect of genotype by classifying alleles according to McKone et al[11]. We grouped the alleles as

"severe" alleles that belong to either class I, II or III, "mild" alleles that belong either to class IV or V and "unclassified" alleles that could not be classified to any of the previous classes (these include the alleles that belong to class Unknown according to McKone et al[11]).
We then grouped the patients according to such genotypes in the following classes: severe/severe (i.e. both alleles belonging to class I, II or III), severe/mild (i.e. one allele belonging to class I, II or III and the other allele belonging to class IV or V), mild/mild (both alleles belonging to class IV or V) or UN (at least one of two alleles unclassified).
The ECFSPR collects the best FEV1 value performed over the year and the corresponding height and weight. Three countries make an exception: France reported the last FEV1 of the year, Germany the value closest to the patient's birthday, and United Kingdom the one at the annual assessment. All FEV1values in litres were transformed into percent of predicted (FEV1%) according to Wang et al[12] for children, and Hankinson et al[13] for adults. We used CDC 2000[14] references for computation of BMI standard deviation scores (SDS) and we categorised them into: $SDS \leq -2$, $-2 < SDS < 2$ and $SDS \geq 2$.

Statistical analysis

We used linear and logistic regressions to investigate association between FEV1% and age, age at diagnosis, sex, pancreatic status, chronic *P. aeruginosa* infection, CF-related diabetes (CFRD), BMI, and genotype. Further details on statistical analyses are provided in the on line supplement.

Role of the funding source

None of the funding sources had a role in study design; in the collection, analysis, and interpretation of data; in writing or reviewing the paper; nor in the decision to submit the paper for publication.

Results

Study population

Data referring to 20204 patients with CF seen in a CF clinic during 2007 were registered in the ECFSPR. Children younger than six years (n=4420) were excluded from the analyses due to their unreliable ability to perform spirometry and to the lack of valid reference values for lung function. Patients who had had a lung transplantation in their life (n=446) were excluded, their FEV1 not representing their disease stage (United Kingdom gave information only on transplants performed during 2007, so patients from UK that had had a lung transplant before 2007 are included). For additional 606 (3·9%) patients, FEV1% could not be computed, because of missing values for FEV1 or height. Our study population finally included 14732 patients (52·8% males).

Table 1 describes the main demographic and clinical characteristics of the included patients. Information on genotype was available for 13613 (92·4%) patients and 8·6% of the alleles remained unidentified after DNA analysis. When classified according to McKone et al.[11], 63.3% of patients carried two alleles that belong to class I or II or III, 5.4% of patients carried one allele that belongs to class I or II or III and the other allele that belongs to class IV or V, 0.2% of the patients carried two alleles that belong to class IV or V. The remaining patients carried at least one allele that could not be classified. When classified according to F508del genotype, 86·7% of patients carried the F508del allele (48·3% in homozygosity and 38·4% in compound heterozygosity). The proportion of patients with low BMI (SDS ≤ -2) increased from children to young adults but decreased again in older patients (after 30 years of age). 86·7% of the patients had pancreatic insufficiency. Prevalence of chronic infection with *P. aeruginosa* gradually increased with age until it reached a plateau of 55% at 25 years of age (Figure 1). CFRD was found in 12·4% of the patients (11·2% of males and 13·7% of females); its prevalence increased with age, from almost absent in children below 10 years to 22·7% in the patients 30+ years old.

FEV1 characteristics

FEV1% was negatively associated with age (Figure 2): mean FEV1% decreases from 91·2% (95% CI: 90·4; 91·9) in the 6-9 year-olds to 55·5% (53·4; 57·6) in the 40-44 year-olds (Table 1). As shown in Figure 2, the decline starts slowly, becomes sharper at age 12, continues until the age of 20 years, and then stays fairly stable. The number of patients (Figure 2, histogram) instead is quite uniform up to age 18 years, when it steadily decreases across the remaining years, although with a less steep decrease from age 30 years. Thus, we noticed a gap of roughly 6 years between the sharper decline in FEV1 and the subsequent decline in the number of patients.

Table 2 shows the differences in the estimated marginal means of FEV1% computed from the regression models: they represent the estimated effect of each factor on FEV1, after adjusting for age and country only (columns 2 and 3) and after adjusting for age, country, genotype, sex, Pancreatic status, CFRD, P. aeruginosa and BMI (columns 4 and 5). Genotype did not show a clinically relevant effect on FEV1: the highest estimated difference after adjusting for age and country effect was between patients with the two severe alleles and patients with one severe and one mild allele, the difference in FEV1 being -10·9 percent points (95%CI: -12·6; -9·1). This difference decreased to -3·8 percent points (95% CI: -5·9; -1·6) after adjusting for the other covariates. Similarly, males had higher FEV1% values than females by 1·1 percent points (95% CI: 0·3; 1·8) after adjusting for age and country effects, and this difference increased to 2·7 percent points (95% CI: 1·8; 3·6) after adjusting also for genotype, BMI, pancreatic status, *P. aeruginosa* infection, and

presence of CFRD. Since sex did not reach the threshold of clinical relevance, it was not included in the final model.

Pancreatic status was associated ($p<0{\cdot}0001$) with FEV1 after adjusting for age and country, but the difference between sufficient and insufficient patients decreased from 12·9 percent points (95% CI: 11·8; 14·2) to 6·7 percent points (95%CI: 5·2; 8·2) after adjusting for sex, genotype, BMI, *P. aeruginosa* infection, and CFRD.

Patients who have CFRD showed FEV1% values 11·6 percent points (95% CI: 10·4; 12·8) lower than patients without CFRD, but this difference decreased to 8 percent points (95% CI: 6·6; 9·5) after additionally adjusting for sex, genotype, BMI, *P. aeruginosa* infection, and pancreatic status.

Chronic infection by *P. aeruginosa* was associated with FEV1 ($p<0{\cdot}0001$) after adjusting for age, country, sex, genotype, BMI, pancreatic status, and presence of CFRD. Infected patients showed, on average, values of FEV1% 13·0 percent points (95% CI: 12·0; 14·0) lower than uninfected patients.

BMI was positively associated with FEV1 ($p<0{\cdot}0001$) after adjusting for age, country, sex, genotype, *P. aeruginosa* infection, pancreatic status, and presence of CFRD. Patients showing a BMI in a normal range (i.e. between -2 and +2 SDS) had, on average, FEV1% 21·6 percent points (95% CI: 20·0; 23·2) higher than patients with poor BMI (≤-2 SDS).

Based on these results, we selected age, BMI, and chronic *P. aeruginosa* as potential modifiers of FEV1 in our final model due to their clinically relevant and their statistically significant effect. We also included pancreatic status and CFRD because, although their adjusted effect did not reach the clinically relevant threshold of 10 percent points, from our data there was strong evidence of a non-negligible effect (approximately 8 percent points). Age at diagnosis had a statistically significant ($p<0{\cdot}0001$) effect on FEV1, after adjusting for all the previously described factors, but it was not clinically relevant and therefore it was not included in the final model: for each additional year of age at diagnosis, patients showed an increased FEV1 value of 0·16 percent points (95% CI 0·10; 0·22). We carried out a sensitivity analysis of the selection of the covariates in the final model changing the minimal threshold of clinical relevance, and any threshold above 5 percent points difference in FEV1 (i.e. the variation that other authors claim exist in FEV1 due merely to different timing of FEV1 measurement) does not change the covariates that were selected in the final model, as can be seen from table 2, column 2.

In this final model we tested for interaction between variables. We found a statistically significant interaction between *P. aeruginosa* infection and pancreatic status ($p=0{\cdot}012$). After adjusting for country, age, BMI and presence of CFRD, pancreatic sufficient patients not infected with P. aeruginosa had, on average, FEV1 values 16·5 percent points higher than infected ones (95%CI: 13·8; 19·2). Among pancreatic insufficient patients, this difference reduced to 12·9 percent points

(95%CI: 11·8; 13·9). Described from another perspective, pancreatic sufficient patients not infected with P.aeruginosa had, on average, FEV1 values 9·0 percent points higher than pancreatic insufficient ones (95% CI: 7·6; 10·5). Among patients infected with P.aeruginosa, this difference was only 5·4 percent points (95% CI: 2·9; 7·9). There was also evidence (p=0·021) of interaction between *P. aeruginosa* infection and CFRD. After adjusting for country, age, BMI and pancreatic status, the difference in FEV1% between infected and uninfected patients without CFRD was 13·8 percent points (95%CI:12·7;14·8) in favour of those uninfected, whereas among patients with CFRD the difference was 10·5 percent points (95%CI: 7·9; 13·1), in favour of those uninfected. While these differences are statistically significant, they are clinically negligible, therefore we did not include any interaction in the final model.

In order to express the magnitude of the effect of the risk factors identified in the previous analyses on severe lung disease we carried out a multiple logistic regression analysis (based on 8650 patients with information on all covariates). Severe lung disease was defined as having FEV1% below 40%, a widely accepted clinical threshold. As shown in Figure 3, the odds of having severe lung disease significantly increases with age (test for quadratic trend p<0·0001). Of the other factors, BMI had the biggest impact on lung disease: patients with poor BMI (i.e. equal to or lower than -2 SDS) experienced a 6-fold increased odds of severe lung disease (OR=6·0, 95% CI 5·0; 7·3) compared to patients with normal BMI (between -2 and +2 SDS). *P. aeruginosa* chronic infection was the factor that had the second biggest impact: being infected increases the odds of severe lung disease by 2·4 (95%CI: 2·0; 2·7). Furthermore, we estimated that pancreatic insufficient patients experienced an odds of severe lung disease twice pancreatic sufficient patients (95%CI: 1·6; 2·5) and patients affected by CFRD had a 1·8 fold increased odds of severe lung disease compared to patients not affected by CFRD (OR=1·8, 95% CI 1·6; 2·2). When we inspected the variation of odds ratios between countries to check for interaction, we concluded that the odds ratios are similar across countries (results not shown).

Discussion

The heterogeneous European CF population showed a wide variability of factors potentially associated with FEV1. We aimed at identifying such factors after adjusting for potential confounders. In this population five factors were associated with poor pulmonary function in patients with CF older than 5 years. Three of the five factors are either partially preventable and/or potentially treatable (poor nutritional status, chronic *P. aeruginosa* infection, and CFRD). The fourth, pancreatic function, indirectly represents the degree of CFTR dysfunction and it might be correctable in the future by therapies that increase the level of functional CFTR. The fifth factor,

age, is challenging since it reflects the continuing exposure to additional deleterious factors that might be preventable or treatable with adherence to the current available therapies.
Low BMI was the strongest potentially preventable factor found in the current study of ECFSPR data. The odds of severe lung disease was six times higher for patients with very poor nutrition than patients with BMI within 'normal' range, the highest OR of all the preventable factors. Although a causal relationship cannot be proved by our study (severe lung disease by itself can cause poor nutrition through increased energy needs, reduced appetite and gastrointestinal involvement like reflux and nausea), we showed that poor BMI was significantly associated with poor lung function, even after taking into account the effect of age, pancreatic status or infection with *P. aeruginosa*. The association between better nutritional status, better pulmonary status and survival has been already demonstrated[15], and aggressive intervention early in life aimed at growth and nutrition may positively affect pulmonary function[16]. However, the current study shows that despite the available guidelines for prevention and correction of nutritional deficiencies in CF[17-18], 9·5% of the patients with CF in Europe still suffer from severe malnutrition (i.e. with a BMI ≤ -2 SDS).

Chronic infection with *P. aeruginosa* was the second factor negatively associated with FEV1. This infection is well-recognized as a cause of morbidity and mortality in CF[19]. In the current study chronic *P. aeruginosa* infection was significantly associated with lower FEV1 after adjusting for age and other potential confounding factors and increased the likelihood of severe lung disease 2·4-fold. This association was also found by both Vandevanter[1] and Konstan[26]. The association seem to be stronger in our study, which could be attributed to the use by both authors of 'pseudomonas at least once' as their definition of infection, whereas we use 'chronic pseudomonas', which usually implies longer exposure and very often mucoid bacteria. Also, both authors investigated children/teenagers, who will have been exposed to the bacteria for shorter time on average, especially in the chronic form, which would also tend to show a weaker association. It is noteworthy though, that even in young patient with only intermittent or early chronic infection, the association is still significant. The importance of the association between *P. aeruginosa* infection and poor lung function is that although most *P. aeruginosa* infections are not acquired in the hospital, acquisition by cross-infection can be prevented using infection control measures. Furthermore, newly acquired infections can be eradicated in over 80% of the cases by antibiotic therapy immediately after the onset of *P. aeruginosa* infection[20]. Once chronically colonized, efforts should be directed towards suppression of the infection. Clinical studies show that inhaled antibiotics improved pulmonary function and reduced the rate of exacerbations in patients already infected with *P. aeruginosa*[21].

CFRD was found in the current study to be associated with lower lung function, also after controlling for age and BMI. It is the most frequent co-morbidity diagnosed today, occurring in up to 40% of adults, 25% of adolescents, and 9% of children[22]. CFRD is associated with a rapid decline in lung function, and increased risk of respiratory failure[23]. Given that CFRD is often clinically silent in the initial stages, it may be valuable to study the potential impact on FEV1 of annual CFRD screening for early diagnosis[17]. The current study confirms the association between CFRD and poor lung function. Since CFRD is currently not preventable, it should be diagnosed as early as possible. Treatment with insulin enhances the nutritional status, temporarily improves pulmonary function and delays the decline in FEV1[24].

Pancreatic status in CF is genetically determined and it is associated with CFTR genotype[25]. Pancreatic sufficiency in CF is associated with carrying at least one CFTR allele from class 4 or 5 CFTR mutations that are associated with the presence of sufficient functional CFTR chloride channels over the apical membrane of the exocrine epithelial cells to maintain adequate pancreatic exocrine function. Compared to patients with PI, patients with PS are diagnosed later, have lower sweat chloride levels, and better nutritional status[25,26]. The current study showed that PS is associated with higher lung function even after controlling for nutrition, infection with *P. aeruginosa,* and CFRD. The importance of this finding is that patients with PS have better pulmonary function independent of other measured variables. A previous study by Corey et al.[27] showed that patients with PS had a lower rate of decline of FEV1. Therefore, for epidemiological studies and bench mark analysis, when comparing pulmonary function, control for pancreatic status is required since the prevalence of PI may differ between cohorts. Pancreatic status (or the associated genotype) is not a preventable or treatable factor; however, emerging new therapies are under development that may recover some of the lost CFTR function, thereby shifting a “severe” CFTR mutation towards a “mild” partially functional mutation[6,7] which is expected to be associated with better pulmonary function.

The ECFSPR data do not give evidence of an association between genotype and FEV1. This is in concordance with previous studies that showed variable lung disease in patients with CF regardless of their genotypes, raising the importance of the other variables previously indicated[25,28].

Since pancreatic status and genotype are strongly related, one would expect an association between FEV1 and genotype as well, but since pancreatic status and genotype convey very similar information, once one covariate is present in the model, the other does not additionally explain much more variation of FEV1.

Although we did not find evidence in our data of the need to control for genotype for epidemiological and comparative studies, more detailed studies are needed to evaluate the importance of genotype on lung function.
In our study, as in other cross-sectional studies, FEV1 seems to be maintained in the advanced age groups (Figure 2). This is potentially a survivor effect: patients with the most severe lung disease are not included in cross-sectional studies because they died or had lung transplant, leaving an increased proportion of "less severely ill" patients in the eldest age groups.

In conclusion, the current study on a large patient population with CF shows the independent effect of nutrition, chronic *P. aeruginosa* infection, and CFRD on FEV1 that currently is the gold standard measure of disease severity. Since these factors are to some degree preventable or are potentially treatable, it emphasizes the importance of early identification of these modifiers of CF lung disease through adequate routine tests. Lowering the rates of new acquisition by *P. aeruginosa* together with methods to suppress its chronic infection, and routine screening for CFRD should be additional therapeutic targets in CF care and be used as benchmark comparisons for standards of care. Furthermore, for epidemiological and clinical studies when comparing groups of patients with CF, it is important to standardize the patients groups not only according to age, but also according to pancreatic and nutritional status, chronic infection by *P. aeruginosa* and CFRD. Since this was an observational study based on registry data, there is the possibility of residual confounding effect of factors that were not measured in the registry.

Conflict of interests

None to declare.

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Authors' contribution

EK, LV, AZ and HVO have been involved in the acquisition of the data, designed the study and wrote the manuscript. LV and AZ performed the statistical analyses. SMN critically revised the statistical analyses in the manuscript and assisted in the preparation of the UK dataset. EH, HE, PD, VG, UK have been involved in the acquisition of the data and revised the manuscript.

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Figure 1 Prevalence of chronic *P. aeruginosa* infection and total number of patients (histogram) by age (in years).

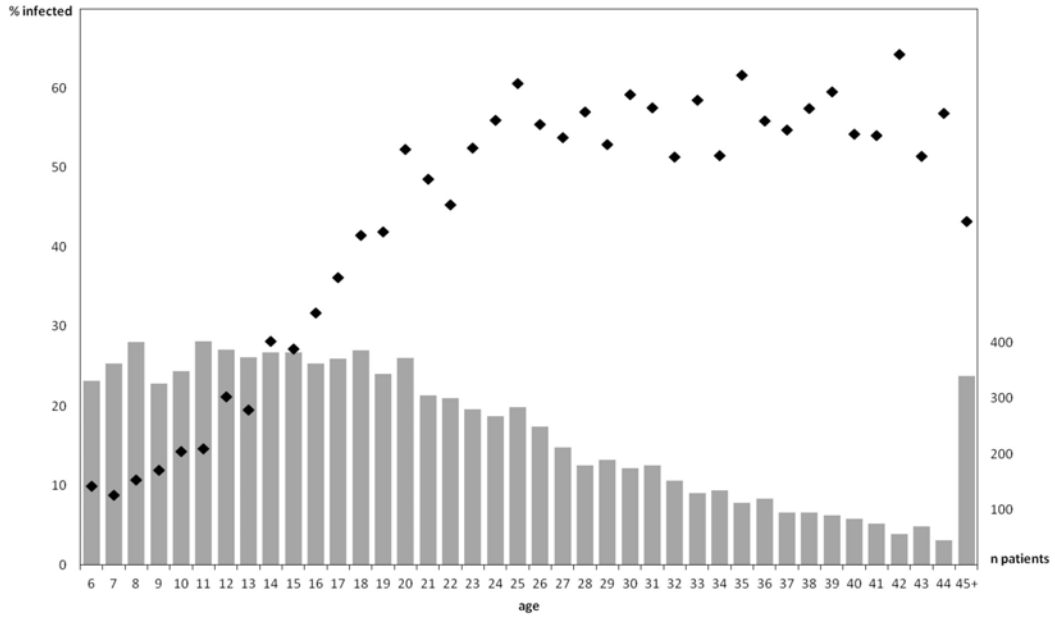

Figure 2 Mean and 95%CI FEV1% and number of patients by age (in years).

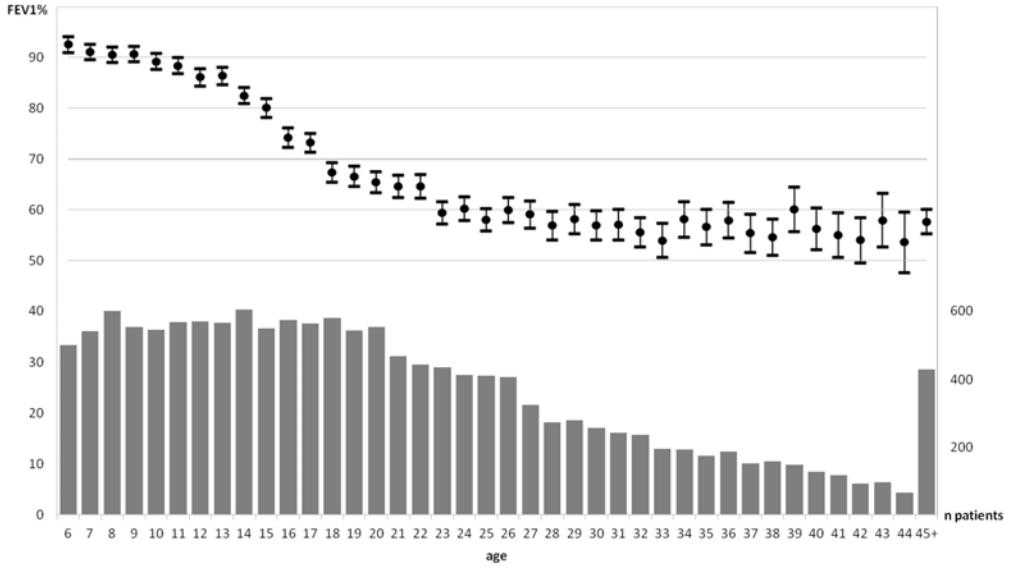

Figure 3 Multiple logistic model: effect of age, BMI, chronic *P. aeruginosa* infection, pancreatic status, and CFRD on the odds of having FEV1<40% of predicted. Estimates are controlled for country. Model estimated on 8,650 patients from Austria, Belgium, Bulgaria, France, Greece, Italy, The Netherlands, Slovenia,
UK.

		n(%) of patients with FEV1<40%pred	OR	(95%CI)
Age (years)	6-9	14/1290 (1.1%)	0.6	(0.3; 1.1)
	10-14[a]	36/1708 (2.1%)	1	-
	15-19	164/1636 (10.0%)	3.8	(2.6; 5.5)
	20-24	299/1359 (22.0%)	8.5	(5.9; 12.2)
	25-29	242/981 (24.7%)	10.0	(6.8; 14.5)
	30-34	207/672 (30.8%)	16.0	(10.9; 23.5)
	35-39	121/442 (27.4%)	14.8	(9.8; 22.4)
	40-44	83/277 (30.0%)	19.2	(12.4; 30.0)
	45+	83/285 (29.1%)	23.4	(14.9; 36.6)
BMI SDS	≤-2	341/766 (44.5%)	6.0	(5.0; 7.3)
	(-2;2)	907/7848 (11.6%)	1	-
	≥2	1/36 (2.8%)	0.4	(0.1; 3.0)
Chronic *P. aeruginosa* infection	not infected	428/5511 (7.8%)	1	-
	infected	821/3139 (26.1%)	2.4	(2.0; 2.7)
Pancreatic status	sufficient	123/1318 (9.3%)	1	-
	insufficient	1126/7332 (15.4%)	2.0	(1.6; 2.5)
CFRD	no	900/7603 (11.8%)	1	-
	yes	349/1047 (33.3%)	1.8	(1.6; 2.2)

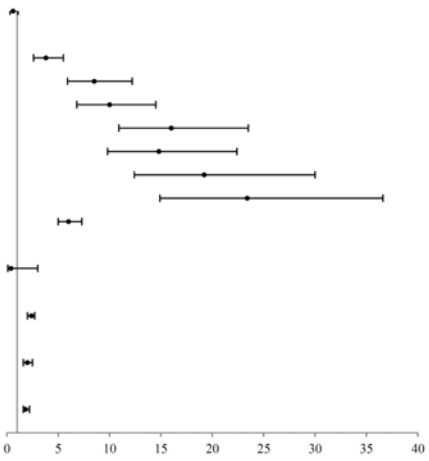

Table 1 Clinical and demographic characteristics of study population (n =14732).

Participating countries, n (%)	
Austria	90 (0.6)
Belgium[a]	757 (5.1)
Bulgaria	23 (0.2)
Czech republic[a]	313 (2.1)
Denmark[a]	320 (2.2)
France[a]	3422 (23.2)
Germany[a]	4133 (28.0)
Greece	56 (0.4)
Israel	390 (2.6)
Italy	632 (4.3)
The Netherlands[a]	871 (5.9)
Portugal	78 (0.5)
Slovenia	37 (0.2)
United Kingdom[a]	3610 (24.5)
Sex, n (%)	
males	7772 (52.8)
Age at FEV1 measurement(years)	
median (range)	19.0 (6-76.7)
n ≥ 18 years (%)	8001 (54.3)
Age at diagnosis (months) (available for 10,994 patients)	
median (range)	7.2 (0[b]-73.3 years)
n ≥ 18 years (%)	668 (6.1)
Genotype[c], n (%)	
patients with DNA analysis	13613 (92.4)
severe/severe	8624 (63.3)
severe/mild	731 (5.4)
mild/mild	27 (0.2)
at least one allele unclassified	4231 (31.1)
unknown alleles	2344 (8.6)
BMI ≤ -2 SDS[d], n (% of age group) (available for 14,625 patients)	
6- 9 years	111 (5.1)
10-14 years	191 (6.7)
15-19 years	316 (11.4)
20-24 years	340 (14.9)
25-29 years	225 (13.3)
30-34 years	104 (9.3)
35-39 years	65 (8.0)
40-44 years	21 (4.2)
45+ years	18 (4.3)
Pancreatic status, n (%) (available for 13,343 patients)	
pancreatic insufficient	11567 (86.7)

Chronic *P. aeruginosa* infection, n (%) (available for 9,748 patients)	
patients with infection	3631 (37.2)
CFRD, n (%) of patients with CFRD (available for 14,013 patients)	
0- 9 years	14 (0.7)
10-19 years	409 (7.6)
20-29 years	697 (18.2)
30 years and above	619 (22.7)
FEV1% of predicted, mean (95%CI)	
6- 9 years	91.2 (90.4-91.9)
10-14 years	86.5 (85.7-87.2)
15-19 years	72.3 (71.4-73.1)
20-24 years	63.0 (62.0-64.0)
25-29 years	58.5 (57.4-59.7)
30-34 years	56.4 (55.0-57.8)
35-39 years	56.9 (55.3-58.6)
40-44 years	55.5 (53.4-57.6)
45+ years	57.7 (55.3-60.0)

[a] Data sent by a national registry

[b] For pre-natal diagnoses, age at diagnosis was set to 0

[c] Alleles classified according to McKone et al[11]:

- severe: alleles that belong to either class I, II or III,
- mild: alleles that belong either to class IV or V,
- unclassified: alleles that could not be classified to any of the previous classes.

[d] SDS: standard deviation score

Table 2 Differences in the estimated marginal means of FEV1% computed from the linear regression models; they represent the estimated effect of each factor on FEV1.

Factor	Difference of adjusted means (95% CI) Adjustment for age and country	p-value	Difference of adjusted means (95% CI) Adjustment for age, country and all other factors present in the table	p-value
Genotype[a]		<0.0001		0.0005
severe/severe – severe/mild	-10.9 (-12.6; -9.1)		-3.8 (-5.9; -1.6)	
severe/severe – mild/mild	-7.1 (-15.7; 1.6)		0.7 (-10.5; 12.0)	
severe/severe – unclassified	-5.7 (-6.5; -4.8)		-2.8 (-3.9; -1.7)	
severe/mild – mild/mild	3.8 (-5.0; 12.6)		4.5 (-6.8; 15.9)	
severe/mild - unclassified	5.2 (3.4; 7.0)		1.0 (-1.1; 3.1)	
mild/mild - unclassified	1.4 (-7.2; 10.0)		-3.5 (-14.8; 7.7)	
Sex				
M – F	1.1 (0.3; 1.8)	0.0051	2.7 (1.8; 3.6)	<0.0001
Pancreatic status				
Sufficient – Insufficient	12.9 (11.8;14.2)	<0.0001	6.7 (5.2; 8.2)	<0.0001
CFRD				
Yes – No	-11.6 (-12.8; -10.4)	<0.0001	-8.0 (-9.5; -6.6)	<0.0001
P. aeruginosa infection				
Yes – No	-15.7 (-16.6; -14.7)	<0.0001	-13.0 (-14.0; -12.0)	<0.0001
BMI[b]		<0.0001		<0.0001
Normal – Poor	23.1 (21.8;24.3)		21.6 (20.0;23.2)	
Above normal – Poor	38.5 (32.8;44.1)		29.1 (21.9;36.2)	
Above normal – Normal	15.4 (9.9;21.0)		7.4 (0.5;14.4)	

[a] Alleles classified according to McKone et al[11]:
- severe: alleles that belong to either class I, II or III,
- mild: alleles that belong either to class IV or V,
- unclassified: alleles that could not be classified to any of the previous classes.

[b] Normal: between -2 and +2 SDS; Poor: below -2 SDS; Above normal: over +2 SDS