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RISK FACTORS FOR *PSEUDOMONAS AERUGINOSA* AIRWAY INFECTION AND LUNG FUNCTION DECLINE IN CHILDREN WITH CYSTIC FIBROSIS

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Highlights:

- 50% of children with cystic fibrosis (CF) acquire *P. aeruginosa* at 5.1 years, and 25% progress to chronic colonisation at 14.7 years
- *P. aeruginosa* chronic colonisation occurs later in life of people with CF in recent cohorts
- Modifier genes influence initial acquisition and chronic colonisation of *P. aeruginosa*
- CF-related diabetes and liver disease are risk factors for *P. aeruginosa* infection
- Decline in ppFEV₁ increases significantly after *P. aeruginosa* infection

ABSTRACT

Background: Cystic fibrosis (CF) lung disease is characterised by recurrent *Pseudomonas aeruginosa* (*Pa*) infections, leading to structural lung damage and decreased survival. The epidemiology of *Pa* infection and its impact on lung function in people with CF (pwCF), especially in recent birth cohorts, remain uncertain.

Methods: We included 1,231 French pwCF under 18 years of age. Age at initial acquisition (*Pa*-IA), chronic colonisation (*Pa*-CC), and duration from *Pa*-IA to *Pa*-CC were estimated using the Kaplan–Meier method. Demographic, clinical, and genetic characteristics were analysed as risk factors for *Pa* infection using Cox regression models. Lung function decline was assessed by modelling percent-predicted forced expiratory volume in 1 s (ppFEV₁) before *Pa* infection, after *Pa*-IA, and after *Pa*-CC.

Results: Among the 1,231 pwCF, 50% had *Pa*-IA by the age of 5.1 years [95% confidence interval (CI) 3.8–6.2] and 25% had *Pa*-CC by the age of 14.7 years (95% CI 12.1 to ∞). We observed that CF-related diabetes and liver disease were risk factors for *Pa*, while gender, *CFTR* variants, and CF centre size were not. Genetic variants of *TNF*, *DCTN4*, *SLC9A3*, and *CAV2* were confirmed to be associated with *Pa*. The annual rate of ppFEV₁ decline before *Pa* was -0.38% predicted/year (95% CI -0.59 to -0.18), which decreased significantly after *Pa*-IA to -0.93% predicted/year (95% CI -1.14 to -0.71) and after *Pa*-CC to -1.51% predicted/year (95% CI -1.86 to -1.16).

Conclusions: We identified and replicated several risk factors associated with *Pa* infection and showed its deleterious impact on lung function in young pwCF. This large-scale study confirmed that *Pa* airway infection is a major determinant of lung disease severity.

1 Introduction

People with cystic fibrosis (pwCF) experience a decrease in lung function over the course of the disease, which is worsened by pulmonary exacerbations (1). End-stage lung disease is caused by structural lung damage due to the inflammation associated with recurrent bacterial infections (2). *Pseudomonas aeruginosa* (*Pa*) is one of the most commonly isolated pathogens in the airways of pwCF. Clinicians closely monitor the occurrence of *Pa*-positive airway cultures, as they may change the lung disease course and requirements for therapeutic care. After *Pa* initial acquisition (*Pa*-IA) and despite effective eradication therapies (inhaled, oral, and/or intravenous antibiotics with success rates ranging from 25% to 100%) (3), persistent infection may occur. This leads to chronic *Pa* colonisation (*Pa*-CC), which is extremely difficult, if not impossible, to eradicate (4). Cystic fibrosis (CF) registries report that 28.5% and 25.1% of pwCF under 18 years of age had positive airway cultures for *Pa* in France and in the US, respectively, while 9.5% and 14% were chronically colonised in France and Europe, respectively (5-7). The prevalence of *Pa* infection has decreased over the years but remains substantial (6, 8). Describing the natural history of *Pa* colonisation and the identification of risk factors may help identify pwCF at most risk of *Pa*-IA and *Pa*-CC for monitoring and therapeutic care adjustment.

Considerable uncertainty remains regarding the epidemiology of *Pa* infections in pwCF. For example, the reported mean age of *Pa*-IA ranges between 1 and 8 years (9-12), and results regarding potential risk factors such as sex, *CFTR* genotype, and meconium ileus vary among studies (11-16). Almost 50% of the phenotypic variability of *Pa* acquisition could be due to genetic modifiers (17) and several studies have identified specific variants as risk factors for *Pa*-IA and *Pa*-CC (18-23). *Pa* infection has also been extensively studied in pwCF to

assess whether it could be associated with a steeper decline in lung function but without consistent results (24-27).

Comparison of results regarding the epidemiology of *Pa*-IA and *Pa*-CC, their risk factors, and their potential association with lung function decline have been difficult because definitions and statistical methods were not the same in the studied CF cohorts (9, 11-16, 24, 25). Moreover, some of these studies involved a limited number of pwCF or a short follow-up duration or are now quite outdated (9, 11, 13, 15, 16). Indeed, in the last decade, standardisation of therapeutic guidelines and organisation of CF care in specialised centres have improved CF data accessibility and quality, allowing new analyses of *Pa* airway infections.

In the present study, we analysed a large French cohort of children with CF to identify risk factors and genetic modifiers for *Pa*-IA, *Pa*-CC, and progression from *Pa*-IA to *Pa*-CC. We modelled longitudinal lung function data and age at *Pa*-IA and *Pa*-CC to precisely estimate the impact of these events on lung function decline.

2 Materials and methods

2.1 Study design and patients

The “French CF modifier gene study” is a nationwide observational study of pwCF in France with prospective data collection from participants. Here we aimed to evaluate the epidemiology of *Pa* infection in pwCF. As of 31 December 2019, 4,961 pwCF, attending one of the 47 French CF centres, were included in this national cohort (corresponding to ~70% of all French pwCF) (6). The study was approved by the French ethical committees (Comité de protection des personnes n°2004/15 and Commission Nationale de L'informatique et des Libertés n°04.404). Written informed consent was obtained from each patient and/or guardian.

Following the adoption of neonatal CF screening in 2001 in France, CF care has been reorganised with the designation of 47 regional expert CF centres, the publication of national recommendations (28, 29) and the standardised prospective collection of longitudinal data. Data from the participating CF centres were collected from electronic or paper medical records and input into a national database for this analysis. All pwCF born between 1 January 2001 and 31 December 2019 were included (age range: 0 to 18 years old, $n = 1,720$). According to the French CF registry, 79.5% of pwCF in this age range consulted at least four times per year in 2018 (2), in agreement with national and international CF care recommendations (29, 30).

We excluded pancreatic-sufficient pwCF ($n = 242$, see Supplementary Information for pancreatic status definition) (31), pwCF with non-European ancestry to homogenise genetic background ($n = 217$), and pwCF for whom phenotypic data were not available due to their recent inclusion ($n = 31$). Finally, 1,231 young pwCF were included in the analyses.

Sex, *CFTR* genotype, meconium ileus, CF-related diabetes (CFRD), and CF-related liver disease (CFLD) were analysed (see Supplementary Information for CFRD and CFLD definitions).

We ranked the CF centres according to the number of pwCF followed-up based on the French national CF registry (6). We defined *small* CF centres as those following less than 125 pwCF (the median number of patients at a reference centre) and *large* CF centres as those following 126–182 pwCF. Among the 47 CF centres, 25 were *small*.

2.2 *Pa-IA, Pa-CC, and time from Pa-IA to Pa-CC*

Microbiological analyses of sputum samples were conducted quarterly in expert laboratories affiliated with CF centres in all pwCF, according to French CF care recommendations (29). Physicians determined the date of *Pa-IA* as the date of the first *Pa* isolation and the date of *Pa-CC* according to the standardised definition: at least three positive samples at least 1 month apart over a 6-month period (29). Age at *Pa-CC* was the age at the third isolation. The duration between *Pa-IA* and *Pa-CC* was also analysed.

2.3 *Lung function*

According to CF care recommendations, lung function testing including spirometry should be performed at every centre visit in pwCF old enough to cooperate (usually ≥ 5 years old) (29, 30). Measurements of the forced expiratory volume in one second (FEV_1) were collected at each visit and expressed as percent-predicted values (pp FEV_1) using the Global Lung Function Initiative equations (32). Measurements of pp FEV_1 of pwCF undergoing *CFTR* modulator therapy, such as ivacaftor and lumacaftor-ivacaftor, were excluded. For pwCF undergoing lung transplantation ($n = 8$), measurements of pp FEV_1 after transplantation were excluded.

2.4 Genotyping

We conducted a replication study of gene variants that had already been shown to be associated with *Pa*-IA and/or *Pa*-CC in pwCF (18-23). Using Kompetitive Allele Specific PCR (LGC Group, Teddington, United Kingdom), we genotyped 14 single nucleotide polymorphisms (SNPs) in or near the following genes: *SLC9A3* (rs57221529, rs4957048), *TNF* (rs1800629, rs361525), *CAV2* (rs8940), *TMC6* (rs34712518, rs2748427, rs12449858), *SLC6A14* (rs3788766), *DCTN4* (rs11954652, rs35772018), and *MBL2* (rs5030737, rs7096206, rs11003125).

2.5 Statistical analysis

Descriptive statistics are reported as mean \pm standard deviation or percentages and 95% confidence intervals (95% CIs), as appropriate. The ages at *Pa*-IA and *Pa*-CC were computed for each person with CF. Those for whom infection or colonisation date was not reported were censored at their age at the last visit. When *Pa*-IA or *Pa*-CC was reported with no age, we considered that it was interval-censored between birth and the age at which it was first mentioned. According to the censoring rules (see Supplementary Information for censoring), 38 (3%) pwCF were interval-censored for *Pa*-IA analysis and 14 (1%) pwCF for *Pa*-CC and duration from *Pa*-IA to *Pa*-CC analysis. We used Cox proportional hazards regression models adapted to interval-censored data to compute hazard ratios (HRs) (33). Cumulative incidence curves, median and quartile ages at *Pa*-IA, *Pa*-CC, and time between infection and colonisation were estimated using the Kaplan–Meier method for interval-censored data.

Covariables changing over time (CFRD and CFLD) were computed by quarter and used in a time-varying Cox analysis.

For the replication genetic association study, we applied additive SNP coding when the SNP minor allele frequency (MAF) was higher than 0.1 and dominant coding otherwise.

Reference alleles were determined as those with the highest frequencies in the European population (<http://www.ensembl.org>). Fisher's exact test was used to test conformance of the allele frequencies with the Hardy–Weinberg equilibrium, and linkage disequilibrium between genetic variants was assessed by computing R^2 and D' .

We used linear mixed model regression to compute the change in ppFEV₁ according to age and *Pa* infection status. For each pwCF, all longitudinal ppFEV₁ data were included in the model. We described individual trajectories of ppFEV₁ by a segmented regression with changes in slope at the age at *Pa*-IA and *Pa*-CC and estimated the average change per year in the periods before *Pa*-IA, between *Pa*-IA and *Pa*-CC, and after *Pa*-CC. More precisely, the model was: $ppFEV_1(a) = b_0 + b_1 \text{ age} + b_2 (\text{age} - \text{age}_{IA})_+ + b_3 (\text{age} - \text{age}_{CC})_+ + \varepsilon$; where b_1 was the slope corresponding to the natural decline of ppFEV₁ with age, b_2 and b_3 were the additional slopes after *Pa*-IA and after *Pa*-CC, $()_+$ was the positive part function, and ε was a normally distributed error. We used random intercepts and slopes to capture the heterogeneity in decline in ppFEV₁ and a first-order autoregressive error to capture residual serial correlation that arises from repeated measurements of the same patient (34).

All analyses were carried out using the R software (version 4.1.0, <http://www.R-project.org/>) using packages “lme4” (35), “nlme” (36), “survival” (37), and “MIICD”.

3 Results

3.1 Study population and age at *Pa* infection

As of 31 December 2019, the mean age of the 1,231 pwCF was 12.9 years (\pm 4.0 years) with an average follow-up time of 11.1 years (\pm 4.2 years) (**Table 1**). Among the 1,231 pwCF analysed, five died and eight had a lung transplant during the study period. *Pa*-IA occurred in 847 children (68.8%) at a median age of 5.1 years (95% CI 3.8 to 6.2) (**Figure 1A** and **Table S1**). By the age of 14.7 years (95% CI 12.1 to ∞), 25% of all pwCF had progressed to *Pa*-CC (**Figure 1A** and **Table S1**). Among the 847 patients with *Pa*-IA, 25% developed *Pa*-CC within 6.9 years (95% CI 4.5 to 9.1) following *Pa*-IA (**Figure 1B** and **Table S1**).

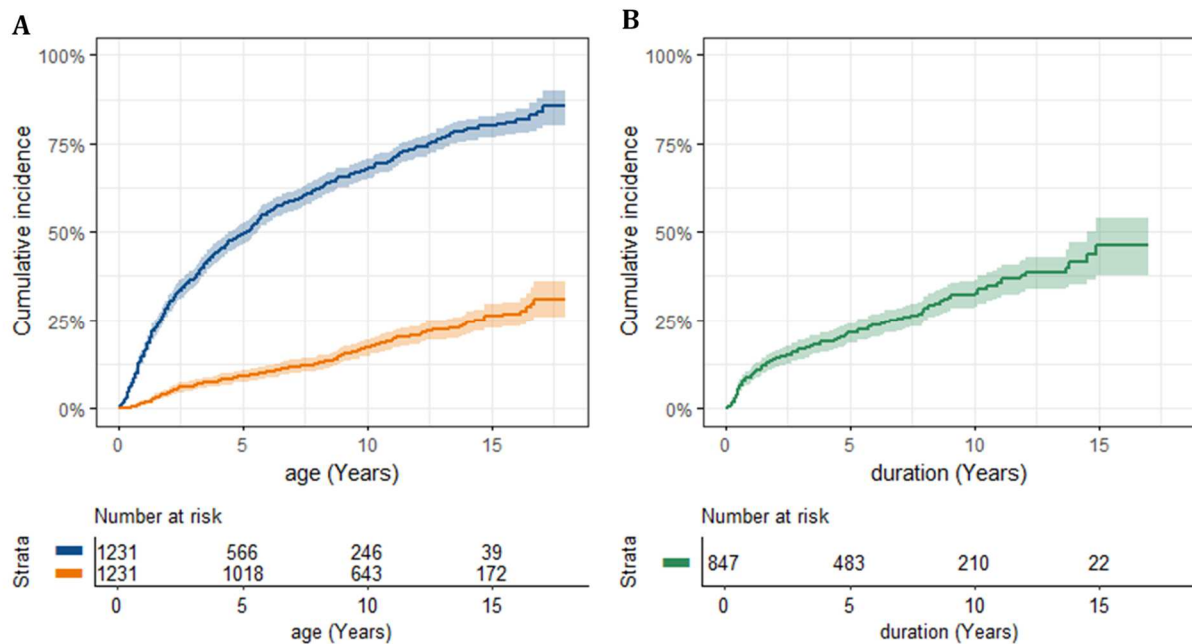
Table 1. Baseline characteristics of the young people with cystic fibrosis

	All young pwCF n = 1,231	Young pwCF with <i>P. aeruginosa</i> initial acquisition n = 847
Males, % (n)	52% (645)	51% (433)
<i>CFTR</i> genotypes, % (n)		
F508del homozygotes	56% (687)	56% (477)
F508del heterozygotes	36% (449)	36% (308)
Others	8% (95)	7% (62)
Meconium ileus, % (n)	20% (242)	19% (159)
Current age* (years), mean \pm SD	12.9 \pm 4.0	13.6 \pm 3.6
Year of birth		
2001–2003	26% (318)	31% (265)
2004–2006	27% (328)	28% (240)
2007–2009	25% (304)	23% (199)
>2009	23% (281)	17% (143)
Age at CF diagnosis (years), mean \pm SD	0.3 \pm 1.0	0.3 \pm 1.0
Follow-up time [†] (years), mean \pm SD	11.1 \pm 4.2	12.0 \pm 3.9
Death, % (n)	0.4% (5)	0.2% (2)
Lung transplant, % (n)	0.6% (8)	0.8% (7)
Number of ppFEV ₁ [‡] measurements per year, mean (95% CI)	3.45 (3.35–3.54)	3.49 (3.37–3.60)

*At December 31, 2019; [†]Time between the birth date and the date of last news; [‡]Forced expiratory volume in one second (FEV₁) measurements were expressed as percent-predicted values (ppFEV₁) using the Global Lung Function Initiative equations (32), number of measurements was computed in the 1100 pwCF aged over 5 years of the lung function decline analysis

Abbreviation: pwCF: people with cystic fibrosis; *CFTR*: cystic fibrosis transmembrane conductance regulator

Figure 1. (A) Cumulative incidence of *Pseudomonas aeruginosa* (*Pa*) initial acquisition (blue) and chronic colonisation (orange) according to age in 1,231 young people with cystic fibrosis (pwCF) born after 2000, with 95% CIs. (B) Cumulative incidence of *Pa* chronic colonisation according to duration after initial acquisition in 847 young pwCF, with 95% CI



3.2 Risk factors for *Pa*-IA and *Pa*-CC

Clinical and demographic risk factors associated with *Pa*-IA, *Pa*-CC, and time between these events are shown in **Table 2**, and median and quartile ages are detailed in **Table S1**. Age at *Pa*-CC increased in pwCF born after 2003 and in the most recent CF cohorts, whereas this was not the case for *Pa*-IA (**Figure S1**). Moreover, pwCF born later had a lower risk of *Pa*-CC than those born between 2001 and 2003, as detailed in **Table 2**. pwCF with CFRD had an increased risk of *Pa*-IA (HR: 2.35, 95% CI: 0.81–6.81) and *Pa*-CC (HR: 1.70, 95% CI: 0.56–5.15) (**Table 2** and **Table S1**).

Young pwCF with CFLD had an increased risk of *Pa*-IA (HR 1.30; 95% CI: 0.98–1.73). The risk was unchanged for *Pa*-CC (HR 1.01; 95% CI: 0.67–1.53) (**Table 2**). Neither sex, *CFTR*

variants, meconium ileus at birth, or the size of the CF centres was associated with age at *Pa*-IA or *Pa*-CC or time in between *Pa*-IA and *Pa*-CC (Table 2).

Table 2. Analyses of clinical and demographical characteristics as risk factors for *Pseudomonas aeruginosa* initial acquisition, chronic colonisation, and duration from initial acquisition to chronic colonisation in 1,231 young people with cystic fibrosis

	Initial acquisition HR (95% CI) *	Chronic colonisation HR (95% CI) *	Duration from initial acquisition to chronic colonisation HR (95% CI) *
Sex			
Female	Reference	Reference	Reference
Male	0.95 (0.83–1.09)	0.85 (0.66–1.10)	0.85 (0.66–1.10)
Year of birth			
2001–2003	Reference	Reference	Reference
2004–2006	0.90 (0.75–1.07)	0.71 (0.51–0.98)	0.78 (0.57–1.07)
2007–2009	0.92 (0.76–1.11)	0.72 (0.50–1.04)	0.82 (0.57–1.16)
>2009	1.00 (0.81–1.24)	0.66 (0.40–1.07)	0.75 (0.47–1.21)
CFTR genotype			
F508del homozygotes	Reference	Reference	Reference
F508del heterozygotes	0.97 (0.84–1.12)	1.01 (0.76–1.33)	1.00 (0.76–1.32)
Others	0.98 (0.75–1.28)	1.35 (0.86–2.12)	1.36 (0.87–2.14)
Meconium ileus			
Absence	Reference	Reference	Reference
Presence	1.06 (0.88–1.28)	0.80 (0.54–1.18)	0.79 (0.53–1.18)
CFRD			
Absence	Reference	Reference	Reference
Presence	2.35 (0.81–6.81)	1.70 (0.56–5.15)	1.33 (0.47–3.79)
CFLD			
Absence	Reference	Reference	Reference
Presence	1.30 (0.98–1.73)	1.01 (0.67–1.53)	0.84 (0.56–1.24)
Size of CF care centre (n)			
Small (≤ 125)	Reference	Reference	Reference
Large (126–282)	1.01 (0.88–1.15)	1.04 (0.81–1.35)	1.04 (0.81–1.35)

*HRs of sex, year of birth, *CFTR* genotype, meconium ileus, and size of CF care centre were computed by Cox proportional hazards regression models adapted for interval-censored data; HRs of CFRD and CFLD were computed by Cox proportional hazards regression models adapted for time-varying covariables

Abbreviations: HR: hazard ratio; *CFTR*: cystic fibrosis transmembrane conductance regulator; CFRD: cystic fibrosis related diabetes; CFLD: cystic fibrosis related liver disease

3.3 Genetic analysis

The results of the SNP replication study are described in Table 3 and illustrated in

Figures S2 to S6, and the median and quartile ages are detailed in Table S2. Briefly, two SNPs

located in *DCTN4* and *TNF* (rs35772018 and rs361525, respectively) were associated with both *Pa*-IA and *Pa*-CC (HRs detailed in **Table 3**; illustrations in **Figures S2** and **S3**).

Two *SLC9A3* SNPs, rs57221529 and rs4957048, were associated with *Pa*-CC and the time between *Pa*-IA and *Pa*-CC (HRs detailed in **Table 3**; illustrations in **Figures S4** and **S5**). They were not in strong linkage disequilibrium ($R^2 = 0.76$, $D' = 0.90$).

CAV2 rs8940 was associated with the duration between *Pa*-IA and *Pa*-CC (HRs detailed in **Table 3** and **Figure S6**). *CAV2* rs8940 MAF was higher in our cohort than in the European population (0.30 vs. 0.16, respectively), with an overrepresentation of the homozygotes for the rare allele (**Table S3**). We found no association between variants of *SLC6A14*, *TMC6*, and *MBL2* and age at *Pa*-IA, *Pa*-CC, or time in between *Pa*-IA and *Pa*-CC (**Table 3**).

Table 3. Association analyses between modifier genes and *Pseudomonas aeruginosa* initial acquisition, chronic colonisation, and duration from initial acquisition to chronic colonisation in 1,231 young people with cystic fibrosis

Gene	Alleles*	Initial acquisition HR (95% CI) **	Chronic colonisation HR (95% CI) **	Duration from initial acquisition to chronic colonisation HR (95% CI) **
<i>CAV2</i>				
rs8940	C/G	1.07 (0.97–1.19)	0.83 (0.68–1.02)	0.79 (0.65–0.96)
<i>SLC9A3</i>				
rs57221529	A/G	1.06 (0.94–1.19)	1.27 (1.03–1.56)	1.27 (1.03–1.56)
rs4957048	A/G	1.10 (0.98–1.24)	1.41 (1.14–1.75)	1.39 (1.12–1.72)
<i>TMC6</i>				
rs34712518†	C/T	1.03 (0.83–1.28)	1.06 (0.71–1.60)	1.06 (0.70–1.59)
rs2748427	A/G	1.08 (0.97–1.21)	0.97 (0.78–1.21)	0.91 (0.73–1.13)
rs12449858†	A/G	0.90 (0.75–1.08)	0.69 (0.47–1.02)	0.73 (0.49–1.08)
<i>SLC6A14</i>				
rs3788766	A/G	1.02 (0.94–1.11)	0.98 (0.84–1.15)	0.96 (0.82–1.12)
<i>DCTN4</i>				
rs11954652†	C/G	1.23 (0.99–1.52)	1.26 (0.85–1.87)	1.11 (0.75–1.64)
rs35772018†	C/T	1.42 (1.03–1.96)	1.81 (1.07–3.07)	1.45 (0.86–2.46)
<i>MBL2</i>				
rs5030737†	A/G	0.83 (0.67–1.02)	0.96 (0.66–1.42)	1.16 (0.79–1.71)
rs7096206	C/G	0.95 (0.84–1.07)	0.94 (0.75–1.18)	0.94 (0.75–1.18)

rs11003125	C/G	0.94 (0.85–1.04)	0.95 (0.79–1.15)	1.02 (0.84–1.23)
TNF				
rs1800629	A/G	0.95 (0.82–1.09)	0.89 (0.67–1.18)	0.93 (0.69–1.24)
rs361525†	A/G	1.31 (1.04–1.65)	1.53 (1.04–2.27)	1.28 (0.87–1.89)

*Minor allele in European Population are in bold; **HRs were computed by Cox regression adapted for interval-censored data, †Dominant model.

Abbreviation: HR: hazard ratio.

3.4 Impact of *Pa* infection on lung function

Among the 1,231 pwCF included, 1,100 had data of ppFEV₁ available after the age of 5 years with an average number of measurements of ppFEV₁ of 3.45 (95% CI 3.35 to 3.54)/year (**Table 1**). The average annual rate of change in ppFEV₁ was -0.38%/year (95% CI: -0.59 to -0.18) in pwCF unaffected by *Pa* or before initial infection. The decrease was faster after *Pa*-IA and after *Pa*-CC (**Table 4**), with an annual rate of change of -0.92%/year (95% CI: -1.14 to -0.71) after *Pa*-IA and of -1.51%/year (95% CI: -1.86 to -1.16) after *Pa*-CC.

Table 4. Lung function decline in people with cystic fibrosis according to *Pseudomonas aeruginosa* infection status

Parameter	Estimate (95% CI)	P-value**
ppFEV ₁ * change rate without <i>Pa</i> / before <i>Pa</i> -IA (%/ year)	-0.38 (-0.59; -0.18)	0.0002
Additional change rate after <i>Pa</i> -IA (%/ year)	-0.54 (-0.78; -0.31)	<0.0001
Additional change rate after <i>Pa</i> -CC (%/year)	-0.59 (-0.96; -0.21)	0.0021

*Forced expiratory volume in one second (FEV₁) measurements were expressed as percent-predicted values (ppFEV₁) using the Global Lung Function Initiative equations (32), allowing adjustment of the model on age, sex, and height.

** For comparison to 0.

Abbreviations: *Pa*-IA: *Pseudomonas aeruginosa* initial acquisition, *Pa*-CC: *Pseudomonas aeruginosa* chronic colonisation

4 Discussion

Over the past three decades, numerous studies have focused on *Pa* infection in pwCF, with varying results (9-16, 24-27, 38). Large studies were scarce and often focus on specific risk factors (14, 16). Here, we provided a description of the course of *Pa*-IA and *Pa*-CC and studied several risk factors simultaneously. We replicated the association of several genetic modifiers (*TNF*, *DCTN4*, *SLC9A3*, and *CAV2*) with *Pa*-IA and/or *Pa*-CC. We also determined lung function decline before and after *Pa*-IA and *Pa*-CC.

The “French CF modifier gene study” includes more than 93% of all French pwCF born after 2001, limiting selection bias (39). We found that *Pa* infection occurred early in childhood, with the median age of patients being 5.1 years at *Pa*-IA. One-quarter of the pwCF were chronically colonised with *Pa* by the age of 14.7 years, within 6.3 years after *Pa*-IA. These results suggest that pwCF in France seem to be at a lesser risk of *Pa* infection than those in the US, as Heltshe *et al.* reported a median patient age at *Pa*-IA of less than 3 years and found that 20% of the pwCF had developed chronic infection by the age of 10 years (10). These differences in age estimates in our cohort could be due to differences in sampling methods and the definition of *Pa*-related events. For example, the French national guidelines recommend collecting sputum, aided by inhalation of aerosolised hypertonic saline in young patients, rather than oropharyngeal swabs (29). Our definition of *Pa*-CC (*i.e.* at least three positive samples at least 1 month apart over a 6-month period) could lead to larger estimates compared to the Leeds Criteria for *Pa*-CC (40, 41). We however did not have access to the raw data to compute the Leeds criteria. Including these definitions in French recommendations would make it easier for future comparisons. We finally acknowledge that deviations from the recommended “sample every quarter to screen for *Pa* infection and

colonisation” would bias the ages at *Pa*-IA and *Pa*-CC upwards, although skipping a few samples should have limited consequences.

We observed that *Pa*-CC occurred later in the most recent birth cohorts, similar to that in American children (8). This may reflect improvements in the standardisation of therapeutic care, especially since the establishment of specialised CF care centres. As of 31 December 2019, the follow-up of the pwCF is still underway; therefore, more pwCF will acquire *Pa*-IA and/or *Pa*-CC later in their life, modifying the age estimates.

Minimal function *CFTR* variants have been associated with the precocity of *Pa* infection (14). As most pancreatic-insufficient pwCF have minimal function *CFTR*, the absence of association between *CFTR* F508del variant and *Pa*-IA or *Pa*-CC that we reported may be expected when analysing only pancreatic-insufficient pwCF. We found that attending large CF centres did not change the risk of *Pa*-IA and *Pa*-CC and neither did gender or meconium ileus. Although statistical significance was not achieved, we found that pwCF with CFRD and/or CFLD had an increased risk of *Pa*-IA and/or *Pa*-CC. Interestingly, several studies reported an association between *Pa* infection and CFRD (42-44). Precisely dissecting the direction of this association could be the object of future studies. Concerning CFLD, the association with *Pa* infection has been little studied; only one study suggested that cirrhosis may increase susceptibility to infection (45). Besides, other variables such as socioeconomic status may change the exposure and course of *Pa* infection in pwCF. Although the French social security system guarantees 100% coverage of healthcare expenses for pwCF, differences in conditions of life and socioeconomic status would be interesting to study in this respect. Also, we could not assess treatment regimens after *Pa*-IA which can delay the age at *Pa*-CC.

We replicated the association of several variants located in genes other than *CFTR* with *Pa* infection. *CAV2* rs8940 was associated with the duration between *Pa*-IA and *Pa*-CC, with a protective effect of the minor allele. A similar trend has been previously reported for *Pa*-CC (21). Genetic linkage between *CAV2* and *CFTR* might explain this association, as the loci are at a distance of only 1 Mb. However, the association between *CAV2* and *Pa*-IA was found to be independent of the *CFTR* variants (21). We replicated the influence of *SLC9A3* (rs4957061) on susceptibility to *Pa*-IA, as previously shown in children with CF (19). We found genetic variants in *SLC9A3* (rs4957048 and rs57221529) involved in *Pa*-CC and the duration from *Pa*-IA to *Pa*-CC. These SNPs have also been described as modifiers of lung function in CF (46) and are located in a solute carrier gene involved in sodium transport. Solute carriers might play an important role in CF pathophysiology by counteracting or worsening the impact of *CFTR* defects on ion and fluid transport across the epithelia, thereby playing a role in *Pa* infection. We did not find an association between *SLC6A14* or *MBL2* and *Pa* infection. The genetic background of pwCF was homogeneous, with both parents with European ancestry. Therefore, population substructure is unlikely to explain the reported associations. Overall, candidate variants were only present in a small proportion of the pwCF (MAF, 0.02–0.30), with fewer contributions to the variability in age at *Pa* infection (HRs from 0.79 to 1.81) and with limited statistical power (see Supplementary Analysis). This was at odds with a twin study in which genetic modifiers explained almost 50% of the variance in *Pa* acquisition (17). Whole-genome analyses, rather than candidate variant approaches, may help reconcile these results.

Finally, we found that the decrease in lung function changed with the *Pa* status. The mean annual decrease in ppFEV₁ prior to *Pa*-IA was 0.38%/year, which increased to 0.93% after *Pa*-IA, and up to 1.50%/year in those with *Pa*-CC. No impact of *Pa*-CC was found in an

analysis of lung disease progression in a cohort of Dutch children with CF (n = 545, age: 11.9 ± 3.8 years) (26). Conversely, Pittman *et al.* compared severe and mild lung phenotypes and found that younger age at *Pa*-IA and *Pa*-CC was associated with lung disease severity (24). Other studies concur with the relationship between *Pa* infection and lung function (25, 27). However, the comparison of the results remains difficult because the analysis methods are different. Here, we used longitudinal measurements of ppFEV₁ of 1100 pwCF, where each person with CF is his or her own control. The linear mixed model allowed the estimation of the impact of *Pa*-IA and *Pa*-CC on lung function. However, we acknowledge that this analysis could only inform the impact of *Pa*-IA and *Pa*-CC that occurred after the first measurement of ppFEV₁, typically between 5 and 6 years of age. Consequently, the estimation of the slope of decline in ppFEV₁ before *Pa* infection was based on pwCF older than 5 years and with a later age at *Pa*-IA. In a recent cohort analysis, the authors modelled ppFEV₁ with age using cubic splines (47). They showed that over the age range of 0–18 years, the decrease in ppFEV₁ was almost linear, supporting our choice in modelling. The acceleration in decline after the establishment of *Pa*-CC underlines the need to delay the progression from *Pa*-IA to *Pa*-CC. As *Pa* infection is a major determinant of lung disease severity in pwCF, preservation of lung function requires intensive monitoring of airway infections from an early stage.

We collected data from paper and electronic patient records. We acknowledge that some information may be missing from these sources, especially in older pwCF. If information was missing in those, though, it would preferentially lead to estimate an older age at infection or colonisation, contrary to observation. This suggests that missing information might not have biased the analysis in this respect. Few pwCF had too little information to date precisely *Pa*-IA or *Pa*-CC; it was 38 pwCF for *Pa*-IA and 14 for *Pa*-CC. A

sensitivity analysis excluding these pwCF showed that the median ages at *Pa*-IA or *Pa*-CC increased by 4–8 months (see Supplementary Analysis).

In conclusion, our study on a large cohort of young pwCF made it possible to determine the clinical and demographic risk factors for *Pa* airway infection. We replicated genetic modifiers for early *Pa*-IA, *Pa*-CC, and short durations between both events. Our results contribute to explaining the variability and implications of *Pa* infection on CF lung function. A thorough understanding and characterisation of the interindividual variability of *Pa* infection will allow the identification of pwCF at risk of developing early *Pa*-IA and/or *Pa*-CC. This is a fundamental step towards predictive and preventive medicine, based on the evaluation of the risks of developing complications and oriented toward the preservation of the pwCF health capital.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest to declare.

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