HEPATOLOGY

HEPATOLOGY, VOL. 69, NO. 4, 2019

Cystic Fibrosis Liver Disease: Outcomes and Risk Factors in a Large Cohort of French Patients

Pierre-Yves Boëlle,[1] Dominique Debray,[2,3] Loic Guillot,[3] Annick Clement,[4,5] and Harriet Corvol,[3,4] on behalf of the French CF Modifier Gene Study Investigators

Cystic fibrosis (CF)–related liver disease (CFLD) is a common symptom in patients with CF. However, its prevalence, risk factors, and evolution are unclear. We analyzed a large database of patients with CF to investigate the incidence of CFLD, its related risk factors, and the use and effect of ursodeoxycholic acid (UDCA) treatment. We retrospectively analyzed 3,328 CF patients with pancreatic insufficiency born after 1985 and recruited into the French CF Modifier Gene Study since 2004. We determined liver status, age at CFLD and severe CFLD onset, sex, *CFTR* genotype, history of meconium ileus, treatment with UDCA, and respiratory and nutritional status. The incidence of CFLD increased by approximately 1% every year, reaching 32.2% by age 25. The incidence of severe CFLD increased only after the age of 5, reaching 10% by age 30. Risk factors for CFLD and severe CFLD were male sex, *CFTR* F508del homozygosity, and history of meconium ileus. Increasingly precocious initiation of UDCA treatment did not change the incidence of severe CFLD. Finally, patients with severe CFLD had worse lung function and nutritional status than other CF patients. *Conclusion:* CFLD occurs not only during childhood but also later in the lifetime of patients with CF; male sex, *CFTR* F508del homozygosity, and history of meconium ileus are independent risk factors for CFLD development; earlier use of UDCA over the last 20 years has not changed the incidence of severe CFLD, leading to questions about the use of this treatment in young children given its possible adverse effects. (Hepatology 2019;69:1648-1656).

SEE EDITORIAL ON PAGE 1379

Cystic fibrosis (CF) is the most common severe autosomal recessive genetic disease in Caucasians. It is caused by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR), a chloride channel expressed in epithelial cells throughout the body.[1] The disease affects several organs such as the lungs, pancreas, intestine, and liver. More than 2,000 mutations in the *CFTR* gene have been described, the most frequent being F508del. The *CFTR* genotype strongly influences pancreatic function, which is either deficient (pancreatic insufficiency [PI]) or normal (pancreatic sufficiency [PS]). It is recognized that patients carrying two severe *CFTR* mutations, also called "CF-causing mutations," have a classical form of CF associated with PI, whereas others have a milder form of disease associated with PS.[2]

Abbreviations: BMI, body mass index; CF, cystic fibrosis; CFLD, CF-related liver disease; CFTR, CF transmembrane conductance regulator; CI, confidence interval; FEV_1, forced expiratory volume in 1 second; HR, hazard ratio; PI, pancreatic insufficiency; PS, pancreatic sufficiency; RMAD, restricted mean age difference; UDCA, ursodeoxycholic acid; US, ultrasonography.

Received August 31, 2017; accepted June 9, 2018.

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.30148/suppinfo.

Supported by Institut National de la Santé et de la Recherche Médicale, Assistance Publique-Hôpitaux de Paris, Sorbonne Université, Agence Nationale de la Recherche (ANR-09-GENO-029), DGS, Association Vaincre La Mucoviscidose, Cystic Fibrosis Foundation (CORVOL16G0), Chancellerie des Universités (Legs Poix), Association Agir Informer Contre la Mucoviscidose, GIS-Institut des Maladies Rares, and Société Française de la Mucoviscidose.

CF-related liver disease (CFLD) includes a wide range of hepatobiliary abnormalities. Focal biliary cirrhosis is the most clinically relevant CFLD because extension of the initially focal fibrogenic process may lead to multilobular biliary cirrhosis with subsequent portal hypertension and related complications.[3] Indeed, multilobular cirrhosis is recognized to have a significant impact on morbidity and accounts for ~2.5% of mortality in patients with CF (the third cause of death after respiratory failure and transplantation-related complications).[4] The prevalence of CFLD remains controversial, with estimates ranging from 2% to 68% in young patients with CF, due to the lack of a consistent definition.[4,5] Identifying patients with CF who are at risk of developing cirrhosis and should undergo regular screening for detection of liver involvement is important as therapeutic interventions are likely to be more effective in patients with early liver disease.[6] However, evidence of CFLD is usually subclinical until the disease is advanced, so CFLD is underdiagnosed. Some risk factors have been recognized as being associated with CFLD, such as severe *CFTR* genotypes and PI, which are dependent upon one another. However, others are still controversial, such as male sex and meconium ileus, a severe neonatal intestinal obstruction occurring in ~15% of patients with CF.[4] Ursodeoxycholic acid (UDCA) is prescribed in CF patients with liver disease, although there is no evidence of an effect.[7]

In this study, we investigated the evolution of liver disease, its related risks factors, and the use and effect of UDCA treatment in a large cohort of patients with CF. CFLD was homogeneously defined using clinical data collected in the French CF Gene Modifier Study, in accordance with the European best-practice guidance.[3]

Patients and Methods

Since 2001, all patients with CF in France are evaluated, at least once a year, by one of the 47 French hospital-based CF centers according to the national CF care recommendations (https://www.has-sante.fr/portail/jcms/c_2792719/fr/mucoviscidose).[8] Neonatal CF screening was generalized in France in 2002.

PATIENTS

Patients with CF treated in 38 participating CF centers between January 2004 and January 2017 were enrolled in the French CF Modifier Gene Study. As of January 1, 2017, 4,798 patients with CF had been included (corresponding to ~80% of all French patients with CF[9]). Longitudinal data were obtained from electronic medical records or abstracted from the patients' paper records, retrospectively before 2004 and prospectively after January 2004. We analyzed patients born after 1985 with PI (n = 3,328) because patients with PS are known to have milder disease[2] (see Supporting Fig. S1 for the flowchart and Supporting Information for pancreatic status definition). Patients born before 1985 were excluded to limit selection biases due to an overrepresentation of patients with milder disease in those surviving longer

View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.30148

Potential conflict of interest: Nothing to report.

ARTICLE INFORMATION:

From the [1]Sorbonne Université, INSERM, Institut Pierre Louis d'épidémiologie et de Santé Publique, IPLESP, APHP, Hôpital Saint-Antoine; [2]Pediatric Hepatology Unit, AP-HP, Hôpital Necker Enfants Malades; [3]Sorbonne Université, INSERM, Centre de Recherche Saint-Antoine, CRSA; [4]Pediatric Pulmonology Department, AP-HP, Hôpital Trousseau; [5]Physiopathologie des Maladies Genetiques d'Expression Pediatrique, Sorbonne Université, INSERM, Paris, France.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Harriet Corvol, M.D., Ph.D.
Centre de Recherche Saint-Antoine, Bâtiment Kourilsky
34 Rue Crozatier
75012 Paris, France
E-mail: harriet.corvol@aphp.fr
Tel: +33-1-44-73-61-74

TABLE 1. Characteristics of Patients With CF at the Time of Their Inclusion in the French CF Modifier Gene Study

Total (n)	3,328
Males, % (n)	52% (1,731)
Current age (years), mean ± SD	15.9 ± 7.7
Year of birth	
1986-1995	36% (1,183)
1996-2005	40% (1,333)
>2005	24% (812)
Age at enrollment (years)	
Birth	35% (1,172)
1-10	36% (1,195)
11-18	29% (961)
European origin, % (n)	89% (2,957)
CF diagnosis <1 year old, % (n)	74% (2,461)
CFTR F508del homozygotes, % (n)	49% (1,645)
Meconium ileus, % (n)	13% (445)

(see Supporting Information). The following patient characteristics were analyzed: sex, *CFTR* genotype (described as homozygous for the *CFTR* F508del mutation, heterozygous for this mutation, or others), and history of meconium ileus (Table 1). Information on liver status, measurements of forced expiratory volume in one second (FEV_1), and body mass index (BMI) were collected at each visit. When no history on CFLD was available in the patient's record, he or she was considered not to be affected. Ages at onset of liver disease and severe liver disease were determined (see definition below). Age at UDCA treatment initiation was also recorded.

CFLD DEFINITION

CFLD was defined according to the European best-practice guidance by Debray et al.[3] when at least two of the following characteristics were present: (1) abnormal physical examination (hepatomegaly and/or splenomegaly); (2) abnormalities of liver function tests defined as an increase of transaminase (alanine aminotransferase and/or aspartate aminotransferase) and/or gamma-glutamyl transpeptidase levels above the upper normal limits (see Supporting Information); (3) ultrasonographic (US) evidence of liver involvement (heterogeneous echogenicity, irregular margins, or nodularity), portal hypertension (splenomegaly, increased thickness of the lesser omentum, spontaneous splenorenal anastomosis, large collateral veins, or ascites), or biliary abnormalities (bile duct dilatation). Patients with cirrhosis, diagnosed by US, computed tomography, and/or magnetic resonance imaging, and/or portal hypertension (splenomegaly, hypersplenism [platelets <150,000 10^9/L and white blood cells <3,000 10^9/L], and/or spontaneous portosystemic shunts on US) and/or esophageal varices were classified as having "severe CFLD."[10]

STATISTICAL ANALYSES

Descriptive statistics used mean ± standard deviation, percentages, and hazard ratios (HRs) with 95% confidence intervals (CIs), as appropriate. The cumulative distribution of CFLD with age was computed using the nonparametric maximum likelihood estimator approach for interval censored data.[11] All patients were considered to be at risk of CFLD since birth. Patients who did not have a CFLD diagnosis before January 2017 were censored at the last visit. In the other patients, the date of diagnosis was computed as the first date when the diagnostic criteria were met based on medical record data. When this date was not precisely known, we considered that it was "interval-censored" between birth and the age at the first report (i.e., CFLD onset could occur at any time in this range—this was the case for 142 out of 605 patients with CFLD [24%]). We defined onset of severe CFLD as the first time cirrhosis, portal hypertension, and/or esophageal varices were reported and applied the same approach regarding uncertainty to this date (the date of severe CFLD was interval-censored in 19 out of 175 patients [11%]). Cumulative incidence curves were compared using the log-rank test adapted to interval-censored data.[11] Factors linked to age at CFLD and the time interval between CFLD and severe CFLD were tested using a Cox-regression model adapted to interval-censored data.[12] CIs were computed by the bootstrap method. We also performed a sensitivity analysis including only patients prospectively enrolled since 2004 (see Supporting Information).

Age at treatment with UDCA was analyzed as above, with patients not under treatment censored at the age of their last visit. We also tested the impact of treatment on the occurrence of severe CFLD. We used instrumental variable analysis to estimate the true causal association between treatment and disease.[13] Indeed, protopathic bias may affect this analysis if UDCA is preferentially prescribed in those who

are more likely to develop the disease,[14] leading to reverse causality with a higher incidence of CFLD in patients under treatment. A statistical instrument must satisfy the following: (1) association with UDCA treatment, (2) changing the risk of severe CFLD only through UDCA prescription (i.e., no direct link between instrument and outcome), and (3) not sharing a common cause with treatment (see Supporting Information). For each analysis, the patients were divided in two groups (those born before and after 1995, in early/late prescribing centers) (see Supporting Information). The effect of UDCA on severe CFLD incidence was computed as the restricted (at age 20) mean age difference (RMAD) at severe CFLD onset, using pseudo-observations for survival and the generalized method of moments.[13]

We computed percent-predicted FEV_1[15] and BMI *Z* scores[16] with respect to those of healthy populations and CF-specific percentiles and *Z* scores for BMI and FEV_1 using previously published methodology.[17,18] BMI and FEV_1 *Z* scores were averaged from measurements taken in the last 3 years for FEV_1 and the last 2 years for BMI and compared according to CFLD stage (no CFLD, CFLD, and severe CFLD) using the Kruskal-Wallis test.

ETHICS

The study was approved by the French ethical committee (CPP 2004/15), and the information collection was approved by the Commission Nationale de L'informatique et des Libertés (04.404). Informed consent in writing was obtained from each patient and/or guardian.

Results

CFLD CUMULATIVE INCIDENCE AND RISK FACTORS

At the time of the study, 18% of the patients had CFLD and 5% had severe CFLD. The incidence of CFLD increased by approximately 1% every year from birth, reaching 32.2% (95% CI, 29.7-35.2) by age 25 and leveling out thereafter (Table 2 and Fig. 1A; Supporting Fig. S4). The most frequent factors defining CFLD were the joint presence of clinical and biochemical abnormalities (50% of the cases), clinical and US abnormalities (26%), and biochemical and US abnormalities (24%). The cumulative incidences of individual items defining CFLD (clinical, biochemical, and US abnormalities) are reported in Supporting Table S2.

The incidence of CFLD was higher in male patients (HR, 1.15; 95% CI, 0.99-1.36), in *CFTR* F508del homozygous patients (HR, 1.17; 95% CI, 1.00-1.37), and in those with a past history of meconium ileus (HR, 1.66; 95% CI, 1.36-2.01) (Table 3; Supporting Fig. S5). The CFLD cumulative incidence at 20 years was 32% in patients with meconium ileus versus 21% in others, 24% in male versus 21% in female patients, and 25% in *CFTR* F508del homozygous versus 20% in other *CFTR* genotypes.

TABLE 2. Cumulative Incidence of CFLD and Severe CFLD

Age	CFLD				Severe CFLD			
(Years)	Number at Risk	Cumulative Number of Events	Average Annual Risk, % (95% CI)	Cumulative Incidence of CFLD, % (95% CI)	Number at Risk	Cumulative Number of Events	Average Annual Risk, % (95% CI)	Cumulative Incidence of Severe CFLD, % (95%CI)
0-5	3,328	94	0.8 (0.6-0.9)	3.7 (3.0-4.5)	3328	13	0.10 (0.06-0.16)	0.5 (0.3-0.8)
5-10	2,978	252	1.3 (1.1-1.6)	9.9 (8.4-10.9)	3053	60	0.39 (0.26-0.48)	2.4 (1.8-3.1)
10-15	2,286	405	1.5 (1.3-1.8)	16.5 (14.8-17.8)	2421	126	0.58 (0.46-0.77)	5.2 (4.4-6.3)
15-20	1,584	519	1.6 (1.3-2.0)	22.9 (20.9-24.7)	1715	153	0.32 (0.23-0.53)	6.7 (6.0-8.3)
20-25	923	599	2.1 (1.6-2.6)	30.6 (28.4-33.0)	1037	173	0.54 (0.28-0.71)	9.2 (7.8-11.0)
25-30	395	605	0.5 (0.1-0.9)	32.2 (29.7-35.2)	471	175	0.22 (0.00-0.67)	10.2 (8.5-12.9)

CFLD was diagnosed when at least two of the following criteria were present: (1) clinical, abnormal physical examination with hepatomegaly and/or splenomegaly; (2) biochemical, abnormal liver function tests; (3) ultrasonographic, evidence of liver involvement, portal hypertension, or biliary abnormalities. Severe CFLD was diagnosed when at least one of the following criteria was present: portal hypertension, esophageal varices, or cirrhosis.

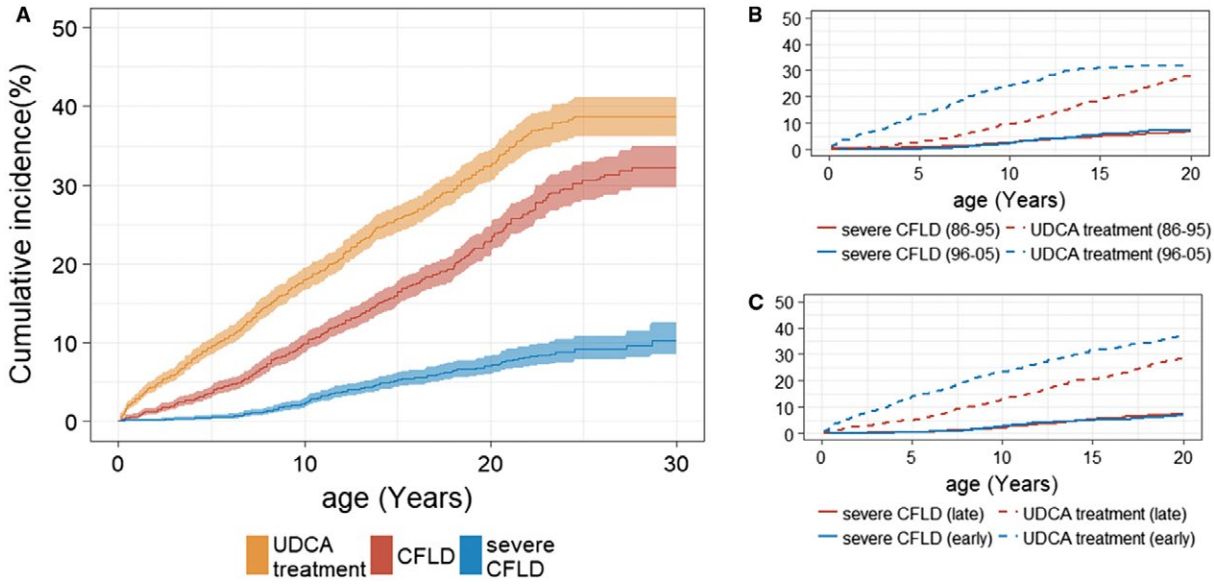

FIG. 1. (A) Cumulative incidence of CFLD, severe CFLD, and UDCA treatment according to age, with 95% CIs. (B,C) Cumulative incidence of severe CFLD (plain) and of UDCA treatment (dotted) in patients with CF born between 1986-1995 and 1995-2005 (B) and in CF centers prescribing UDCA early and late (C).

TABLE 3. Risk Factors for CFLD and Severe CFLD

Patients' Characteristics	Univariable Analysis	Multivariable Analysis*
	HR (95% CI)	HR (95% CI)
CFLD		
Male sex	1.15 (0.99-1.36)	1.15 (0.99-1.37)
Meconium ileus	1.66 (1.36-2.01)	1.64 (1.34-2.01)
CFTR F508del homozygous	1.17 (1.00-1.37)	1.14 (0.97-1.33)
Severe CFLD		
Male sex	1.48 (1.10-2.09)	1.48 (1.1-2.08)
Meconium ileus	1.28 (0.78-1.72)	1.28 (0.77-1.70)
CFTR F508del homozygous	1.10 (0.82-1.48)	1.09 (0.80-1.48)

*In the multivariable analysis, each variable was adjusted for all variables reported in the table.

A multivariable analysis showed that these factors were independently associated with time to CFLD occurrence. Male *CFTR* F508del homozygous patients with meconium ileus (~4% of the population) were the most at risk of developing CFLD with a 33% cumulative incidence at age 20, while the least at risk were non-*CFTR* F508del homozygous female patients without meconium ileus (~21% of the population) who reached 17% cumulative incidence at the same age (Supporting Fig. S6). The sensitivity analysis of patients prospectively enrolled since 2004 showed similar results (see Supporting Fig. S7 and Table S6).

SEVERE CFLD AND RISK FACTORS

Severe CFLD complications were rare up to the age of 5 (Table 2). Overall, the incidence of severe CFLD increased by approximately 0.4% per year after the age of 5, reaching 10.2% (95% CI, 8.5%-12.9%) by age 25 (Table 2 and Fig. 1A; Supporting Fig. S4). Severe CFLD was diagnosed by cirrhosis (56%), portal hypertension (41%), or esophageal varices (3%). Risk factors for severe CFLD were similar to those for CFLD (Table 3). At age 20, the incidence of severe CFLD was higher in male patients (8.5% versus 5.4%), in patients homozygous for the

CFTR F508del mutation (8% versus 6%), and in those affected by meconium ileus (8% versus 7%) (Supporting Fig. S5).

TREATMENT WITH UDCA

Treatment with UDCA increased with age (Fig. 1A). At the age of 30, 38% of all CF patients were under treatment. Treatment with UDCA had been initiated before diagnosis of CFLD in 83% of those treated. UDCA prescriptions changed with time and according to CF center. Patients born after 1995 received UDCA earlier than those born before (Fig. 1B): at 10 years of age, 7% of patients born between 1986 and 1995 were treated with UDCA compared with 23% of patients born after 1995. CF centers could also be split as early or late UDCA prescribers (Fig. 1C). Yet, irrespective of the split, the cumulative incidence of severe CFLD remained the same between groups (Fig. 1B,C). There was no evidence of a change in mean age at severe CFLD onset with UDCA treatment using birth cohort as the instrument (RMAD, 2 ± 3.5 years, P = 0.56 for comparison to 0), using CF center as the instrument (RMAD, 0 ± 1.0, P = 0.98), or combining the two (RMAD, 0 ± 0.8, P = 0.95; see Supporting Information for a detailed description of this analysis).

BMI AND FEV_1 CHANGE ACCORDING TO CFLD AND SEVERE CFLD

At the time of the study, BMI and FEV_1 measurements were available for most patients (Table 4). As expected, patients had impaired lung function, with percent predicted FEV_1 of 75% of the reference population in the non-CFLD group, 71% in those with CFLD, and 63% in those with severe CFLD (P < 0.001). With reference to CF patients, those without CFLD had slightly better lung function (CF-specific FEV_1 percentile 53%) and those with severe CFLD slightly worse (CF-specific FEV_1 percentile 47%) (P = 0.04).

BMI measurements showed the same trend, with CF patients having an altered nutritional status compared with the normal population and increasingly worse performance in those affected with CFLD and severe CFLD (P < 0.001 and P = 0.01 for BMI Z score and CF-specific BMI percentile, respectively). Similar results were obtained in a subset of patients exactly matched for age and sex (see Supporting Information and Table S5).

Discussion

The French CF Modifier Gene Study provided an unprecedentedly large database of 3,328 PI patients with CF born after 1985, enabling us to study the incidence of CFLD and severe CFLD. We observed that CFLD increased with age up to 32% by age 25 and increased with independent risk factors such as male sex, *CFTR* F508del homozygosity, and a history of meconium ileus at birth. We showed that severe CFLD was rare before age 5, increased to 10.2% by age 30 with risk factors similar to CFLD, and was not modified by UDCA treatment. Interestingly, we also found that liver disease was associated with worse lung function and nutritional status.

While CFLD is often believed to develop during childhood, we observed that the incidence rates continued to increase in young adults. CFLD incidence

TABLE 4. BMI and FEV_1 According to CFLD Status

	No CFLD (n = 2,723)	CFLD (n = 431)	Severe CFLD (n = 174)	*P*
Age (years), mean ± SD	15.7 ± 6.5	16.9 ± 6.2	18.9 ± 5.3	<0.001
Male sex	51%	52%	61%	0.036
FEV_1 percent predicted* (mean ± SD)	75.1 ± 23.6	71.0 ± 23.9	62.5 ± 22.7	<0.001
BMI *Z* score† (mean ± SD)	−0.48 ± 0.97	−0.57 ± 1.00	−0.92 ± 0.95	<0.001
CF-specific FEV_1 percentile‡ (mean ± SD)	53 ± 26	51 ± 20	47 ± 26	0.004
CF-specific BMI percentile (mean ± SD)	48 ± 26	47 ± 27	39 ± 24	0.01

*Global Lung Function Initiative equations.[14]
†WHO2007 reference.[16]
‡FEV_1 and BMI *Z* scores were averaged over measurements of the last 3 and 2 years, respectively.

increased between 1% and 2% every year from birth up to the age of 25, reaching 32.2% at this age, at which it plateaued, which may be because of fewer older participants in our study. So far, few studies have reported on CFLD incidence after infancy. In a cohort of 241 young patients with CF followed up for a mean duration of 9.8 years (5 months to 20.6 years), CFLD was mostly diagnosed before 12 years of age but not thereafter.[19] In a cohort of 177 patients, Colombo et al. observed a higher incidence of CFLD than that in the present study (1.8% per year overall), but a plateau was reached at 20 years for male patients and at 10 years for female patients.[20] These two studies included a limited number of patients, mostly children, whereas we were able to analyze a very large cohort of patients with CF representative of all patients under clinical care. Furthermore, in these two studies, patients were only followed up over a short time, meaning that limited information was available to estimate CFLD incidence over the patients' lifetime. In accordance with our findings, a recent study reported that adult-onset CFLD was likely underestimated.[21] The improved survival of patients with CF in the last decade may also explain the change in the incidence profiles of CFLD.[22]

Severe CFLD complications were rare before 5 years of age but increased afterward by 0.3% per year, reaching 10.2% by the age of 30. The prevalence of severe CFLD was higher in male patients, in patients homozygous for the *CFTR* F508del mutation, and in patients with a history of meconium ileus at birth. An international study based on 561 CF patients with severe CFLD (cirrhosis and portal hypertension) showed similar results.[23] The complications were mostly cirrhosis and portal hypertension, as shown by others.[4,24-26] As recently described, we also observed a subset of patients with portal hypertension but no cirrhosis.[26,27] Differences in biochemical characteristics in patients with CFLD and severe CFLD were consistent with aggravation of the disease (Supporting Table S4).

We identified three independent risk factors associated with CFLD and severe CFLD: male sex, *CFTR* F508del homozygosity, and history of meconium ileus at birth. One third of the patients at most risk (*CFTR* F508del homozygous male patients with a history of meconium ileus) developed CFLD by the age of 20, while only 16% of the lower-risk factor group (non-*CFTR* F508del homozygous female patients without meconium ileus) had developed CFLD at the same age. While several studies reported similar associations between CFLD and severe *CFTR* genotype,[19,20,28,29] male sex,[20,28-31] and meconium ileus,[5,19,20,28,29,32] some discrepancies exist.[33,34] So far, the literature is inconclusive about the impact of CFLD on CF evolution. While some studies have shown that patients with CFLD were likely to have a more severe CF phenotype with altered nutrition and lung function statuses,[5,35-38] others did not observe any association.[19,20,34,39,40] We were able to confirm in this large cohort that patients with CFLD had more severe lung disease, with the worst sufferers being patients with severe CFLD complications. Nutritional status showed the same trend. One particularity of this study was the use of CF-specific percentiles and *Z* scores for BMI and FEV_1 that allowed referencing of the patients with CF with respect to their peers and, thus, peer-to-peer comparisons.[17,18]

Finally, we did not observe less severe CFLD as a result of the increasingly precocious use of UDCA in time. UDCA efficacy in CFLD remains controversial.[7] In sharp contrast with pulmonary and nutritional CF complications, where new treatments have been shown to improve survival and quality of life, no drugs have demonstrated an effect on CFLD. Although a recent Cochrane systematic review concluded that "There is currently insufficient evidence to justify routine [UDCA] use in cystic fibrosis,"[7] several guidelines for CFLD patient care still recommend its use.[8,10] High doses of UDCA treatment have even been detrimental to patients with primary sclerosing cholangitis as its biotransformation in the colon produced lithocholic acid, a secondary hydrophobic bile acid with potential toxicity.[41,42] However, this was not seen in CF patients in whom the prescribed doses of UDCA are much lower.[43] In our cohort, UDCA treatment had been initiated increasingly early in CF patients over the last decades, even before the onset of CFLD. In turn, one would expect the occurrence of severe CFLD to decrease or be delayed in patients who are treated earlier in life. The same trend would be expected in centers where treatment is given earlier in life. Yet, the impact of preventive treatments requires taking into account protopathic bias, whereby treatment can be preferentially prescribed to those who are at increased risk of developing the disease. This could cause CFLD incidence to be larger in those treated, actually reversing the cause and effect

and distorting the conclusion.[14] To avoid this bias, we used an instrumental analysis, with birth cohorts and early/late prescribing centers as instruments. We found no changes between early and late treatment with UDCA, based on birth periods or on centers. Such results would be expected in case UDCA treatment has little or no effect on CFLD complications. We, however, acknowledge that an effect of UDCA could be masked in the following situations. First, a delay in severe CFLD symptoms induced by UDCA treatment could have been balanced out by a simultaneous earlier recognition of severe CFLD in the most recent cohort. It is, however, unlikely that severe CFLD symptoms would be missed in CF patients born since the 1980s. Furthermore, a similar, and synchronous, mechanism would have to explain the absence of difference between early- and late-prescribing CF centers. Second, and more subtly, the use of UDCA could be already large and early enough in CF patients so that severe CFLD would be prevented in all those who respond to UDCA and leave only those who do not respond to the treatment as severe cases. In this case, the observed severe CFLD cases would be all such "nonresponders" and, provided their fraction is constant across time and centers, explain the absence of difference in severe CFLD incidence. This would not preclude the existence of true "UDCA responders" actually protected from severe CFLD and would change our conclusion to the absence of treating earlier patients who were not protected with late UDCA treatment.

Other limitations in our analysis mainly concern its design, where data for the years before 2004 were obtained by retrospective examination of medical records. On the basis of CF mortality rate, we have estimated that between 2% and 7% of CF patients born between 1986 and 2004 were not included in our study, which could lead to selection by survival (see Supporting Information). It is also possible that CF clinicians grew more aware of CFLD with time, especially after the study by Colombo et al. in 2002 wherein the diagnostic criteria of CFLD were formalized.[20] Analysis of CFLD incidence in the post-2004 cohort hints that the disease is reported earlier, with a cumulative incidence of 13% (10.5%-15.7%) at 10 years of age versus 10% (8.7%-11.0%) in the whole cohort (see Supporting Fig. S7 and Table S6). As mentioned above, this is less likely to be the case for severe CFLD because the clinical signs are less dependent on interpretation (i.e., cirrhosis, portal hypertension, esophageal varices) and its incidence has not changed over the last 20 years. This is why we focused on severe CFLD in the UDCA treatment analysis.

To conclude, we observed, in this large CF cohort, a high incidence of CFLD. While liver disease is thought to develop at pediatric age in patients with CF, we were able to show that its incidence continuously increased over time, with a consequential rate of progression to severity. CFLD and severe CFLD were associated with male sex, *CFTR* F508del homozygosity, and history of meconium ileus at birth, as well as with worse lung function and nutritional status. Finally, the absence of effect of UDCA treatment on the incidence of severe liver disease is an important finding as UDCA is commonly prescribed in young patients. In the future, it will be critical that potential therapeutic agents be evaluated in well-designed randomized clinical studies, to ensure that the patients most likely to benefit from the treatment are identified.

Acknowledgment: We thank the clinical research assistants involved in the data collection: Pauline Touche, Tania Kombi, and Julie Mesinele. We especially thank the patients and families who participated in this study and the contributing French CF centers (see the list of investigators in Supporting Information).

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Author names in bold designate shared co-first authorship.

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