SUPPLEMENTARY APPENDIX

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Background on CF Centers in France

CF centers were created in France in 2001. These are hospital-based reference centers for the follow-up of patients, in pediatric and adult hospitals. There are 47 CF centers in France to ensure standard of care for all the patients with CF. National recommendations on core CF management are in use in CF centers (https://www.has-sante.fr/portail/jcms/c_2792719/fr/mucoviscidose) (1). Routine neonatal screening for CF was nationally adopted in 2002, but was carried out before in regions with large prevalence.

Definitions

Pancreatic status

Patients were considered to be pancreatic insufficient (PI) if treated for pancreatic insufficiency and/or if their alleles or combination of alleles at the *CFTR* locus were associated with PI in the CFTR2 database (https://www.cftr2.org/). In cases where a mutation was not found (<10), patients were considered PI if the other *CFTR* allele was associated with PI and if they were treated for pancreatic insufficiency. Two patients had no information on *CFTR* genotype and were not considered to be PI and, therefore, were excluded from further analyses.

Abnormalities in liver function tests

Gamma-glutamyl transpeptidase (GGT) levels were considered elevated if >55 IU/L for men and >45 IU/L in women. The thresholds were reduced by 20% in patients younger than 15 years.

Bilirubin levels were considered elevated if total bilirubin level was >17 µmol/L.

Aspartate aminotransferase (ASAT) levels were considered elevated if >60 IU/L in men and > 50 IU/L in women (*i.e.,* more than twice the normal).

Alanine aminotransferases (ALAT) were considered elevated if >70 IU/L in men and > 50 IU/L in women.

Selection bias

CF patients born after 2004 were included at birth, since routine neonatal screening was adopted in 2002 in France. Patients born before 2004 were included in the study if they were alive and consulting for CF in 2004. Here, we present estimates of the number of missing patients among those born between 1986 and 2003. Mortality estimates for CF patients were obtained from the French CF registry (**Figure S2**) (2). **Table S1** shows the number of CF patients included in the study, along with the number of live births in France in the same period taken from national statistics. On the basis of these figures, we postulated that the rate of CF at birth in the population attending the participating CF centers could range between 1.6 and 1.9/10,000 with a "worst case" scenario at 1.9/10,000 and an "average case" scenario at 1.75/10,000. Details of these computations are presented in **Table S1**, with the following conclusions:

- In the "average case" scenario, 2% of all patients born between 1986 and 2003 would be missing.
- In the "worst case" scenario, 7% of all patients born between 1986 and 2003 would be missing.

The average case scenario is remarkably consistent with selection by survival over the first 20 years of life; the worst case scenario implies that up to 5% more patients would be missing from follow-up.

Such differential survival could bias the assessment of risk factors for CFLD development, if the reasons for shorter survival also affect the incidence of liver disease. Liver disease by itself is a rare cause of death in CF, although the third in importance. Indeed, between 2007 and 2010 in France, only 6 deaths out of 256 were attributed to liver disease (3). Most deaths are due to respiratory disease, and there is no strong evidence that the risk factors known for CF liver disease (male sex, meconium Ileus, F508del homozygous) also affect respiratory disease.

Statistical analyses

Interval censoring in CFLD diagnosis

The following diagram shows the different cases for the definition of age at CFLD.

Symbols are: date of (B)irth, date of (C)FLD diagnosis, and date of first (H)ospital visit and (L)ast visit.

1 – Observed: B----------H----------(>1 yr)----------C----------L

In this case, the first mention of CFLD diagnosis in the medical record is more than 1 year after the patient was first seen in the hospital CF center, according to available medical records. Age at CFLD is considered to be age at date (C).

2 – Right-censored: B----------H----------L------C

In this case, the patient was followed up until date L without any mention of CFLD diagnosis. This patient is considered not CFLD at age at date (L).

3 – Interval-censored: B----------H—(<1yr)---C----------L

In this case, the first mention of CFLD is less than 1 year after the first hospital visit. In this case, we consider that CFLD diagnosis could have been made before, had the patient reported to the hospital earlier. We therefore consider that age at CFLD diagnosis is imprecisely known, and may have occurred at any time between birth (B) and age at (C).

Incidence of items defining CFLD and severe CFLD

As recalled above, CFLD is defined when 2 characteristics out of 3 are present in a patient. We computed the cumulative incidence of each item: clinical, biochemical, or ultrasonographic abnormality. Likewise, severe CFLD occurs when either cirrhosis, portal hypertension or esophageal varices are present. We computed the cumulative incidence of each sign reported in **Table S2**. For CFLD, the most prevalent signs were biochemical abnormalities, which were present early in life. Clinical abnormalities were second in occurrence, followed by ultrasonographic abnormalities. In severe CFLD, severe cirrhosis was the first complication to occur, but the most prevalent, at any given

age, was portal hypertension. This means that portal hypertension was often cirrhotic but also non-cirrhotic, as recently described (4, 5).

Ursodeoxycholic acid (UDCA) treatment and the occurrence of severe cystic fibrosis liver disease

Here, we only considered the 2,516 PI patients born from 1986 to 2005. An approach to the analysis of the impact of ursodeoxycholic acid (UDCA) was to study the incidence of severe cystic fibrosis related liver disease (CFLD) as a function of treatment exposure. We found that 776 patients out of the 2516 were treated and 168 (6.7%) had severe CFLD. Among the treated patients, 41 started UDCA treatment after severe CFLD onset, 92 started UDCA and progressed to severe CFLD and 735 were under UDCA treatment without severe CFLD during their follow-up. Analyzing time to severe CFLD with UDCA treatment as a time dependent exposure with a Cox proportional hazard model, we found that the HR for UDCA treatment showed a faster progression to severe CFLD in the treated (HR=7 [CI: 5-9.8]). However, it is likely that UDCA treatment was started earlier in patients who were more likely to develop CFLD according to preliminary signs of disease or with 'at-risk' characteristics such as meconium ileus. This is known as "protopathic bias" where patients with a higher chance of disease are preferentially treated. In such cases, cause and effect can be seemingly reversed in time, leading time-dependent analyses to conclude at an increase in risk in those under treatment. Using a time lag in exposure (for example, counting exposure only after 1 year of treatment) may correct for this bias, however no conventional statistical adjustment method can eliminate this bias. Here, using a 1-year lag led to a reduction in the HR (=5.7), however, it still indicated an increased risk in those treated with UDCA.

We therefore decided to use an instrumental variable analysis. Instrumental variable analyses allow for the analysis of observational data in the presence of unobserved confounding factors. An instrument is a characteristic with the following properties: (1) It is associated with

exposure, (2) it is independent of other confusion factors, and (3) it must change the outcome only through exposure (i.e. it does not have a direct effect on the outcome). Only (1) can be tested from the data; propositions (2) and (3) must be hypothesized. The use of instrumental variables in time of event analysis requires special statistical procedures, for example the use of pseudo-observations (6).

We examined two different instruments to identify the role of UDCA in severe CFLD: birth cohort and CF center, and both together.

Birth cohort as an instrument

First requirement: In recent years, UDCA has been prescribed earlier in CF patients, as shown in **Figure 1B** of the main manuscript. Birth cohort is therefore associated with exposure to UDCA, satisfying the first requirement of an instrument.

Second requirement: Known risk factors for CFLD include male sex, meconium ileus, and *CFTR* genotype. These were confirmed in our analysis, although with small effects. These factors did not change according to birth cohort, as seen in **Table S3**. In other words, these effects could not confound the association between period and UDCA treatment. Birth cohort was therefore not associated with the other risk factors of severe CFLD, satisfying the second requirement for an instrument.

Third requirement: We hypothesized that birth cohort was associated with severe CFLD only through changes in UDCA treatment to satisfy the third requirement. As discussed below and in the manuscript, this assumption may be challenged if one assumes that birth cohort was also linked with increased and earlier recognition of severe CFLD.

In the instrumental analysis of time to event analysis, the effect of exposure (UDCA) can be summarized as the counterfactual difference in the cumulative incidence of event (severe CFLD) at a given age or in the age difference at event (severe CFLD) between exposure and non-exposure (restricted mean age difference at event). In other words, one estimates the difference in age at

severe CFLD onset for the same patient in case he would have been treated versus never treated. Here, UDCA treatment was coded as "present" if prescribed before severe CFLD.

The analysis yielded a difference in age at severe CFLD by 2 ± 3.5 years in case of treatment, but this was not different from 0 (P=0.56).

To check whether it was possible that better recognition of severe CFLD with time could explain our results, we restricted the analysis to patients born between 1990 and 2000 to minimize changes in severe CFLD awareness. The results were once more inconclusive, with a restricted (at age 15) mean age difference of -1.8 ± 7.9 years in case of treatment, P=0.82).

CF center as an instrument

Age at UDCA prescription also changes with CF center. This may be because of the patients' health status and also because of the physician's preference. Here, we used the Kaplan-Meier analysis of age at UDCA treatment according to CF center, and split CF patients according to centers where treatment was prescribed "early" and those in which it was "late" (**Figure S3**). Our definition of "early"/"late" was data-driven, based on making two groups with similar number of patients. We split centers according to their UDCA prescription rate in 5 years old CF patients, defining "early" when the rate was >5% and "late" otherwise. Splitting in 2 groups based on prescription rates at age 10 led to similar results.

We then computed the cumulative incidence of severe CFLD according to early/late prescription (*see* **Figure 1C** in the main manuscript). There was a difference in UDCA prescription in terms of age (P< 0.0001). However, the severe CFLD cumulative incidence curves did not suggest a strong difference (P=0.8). Likewise, the instrumental analysis, using "early" and "late" prescribers reported a difference in restricted (at age 20) age at severe CFLD of 0 ± 1 years (P=0.98 for a difference from 0).

Birth cohort and CF center as instruments

Instruments can be combined in the analysis to reduce variance in the results. Here, pooling the two analyses resulted in a difference in restricted mean (at age 20) in age at severe CFLD of 0 ± 0.8 years (P=0.95 for comparison to 0), which is once more compatible with a limited impact of UDCA treatment on severe CFLD development.

Supplementary analyses

Biochemical characteristics in patients with CFLD and severe CFLD

Levels of the different parameters (ASAT, ALAT, GGT, APRI score, bilirubin and platelet count) over the year before CFLD diagnosis and over the year before severe CFLD diagnosis were all consistent with an aggravation of the disease (**Table S4**).

Body mass index and forced expiratory volume in 1 second in age/sex matched CF-patients

Although we used CF-specific percentiles that account for age and sex differences between patients, we report here comparisons of body mass index (BMI) and forced expiratory volume in 1 second (FEV_1) values in a subset of patients matched for age and sex. The same pattern was present in these age and sex matched patients: FEV_1 was lower in patients with CFLD, and there was a trend for a decrease in BMI with increasing severity that did not reach significance here (**Table S5**).

Patients included after 2004

We analyzed the 1,113 patients born after 2014 and followed from birth in our cohort. Obviously, follow-up was more limited, as all patients were less than 13 years old in the end. The cumulative incidences of UDCA treatment, of CFLD, and severe CFLD are presented in **Figure S7** and **Table S6**. Comparing the whole cohort to patients born after 2004 suggests that UDCA treatment may have been given earlier in recent years, with 13% being treated at age 5 versus 10% in the whole cohort, and 19% versus 18% at age 10. Whereas the cumulative incidence of CFLD was slightly higher in the more recent cohort at the same age, the cumulative incidence of severe CFLD was approximately the same. Altogether, findings of this sensitivity analysis were consistent with the results of the whole cohort.

Supplementary tables

Table S1: Number of patients in the cystic fibrosis (CF) modifier gene study, number of live births in France, and their ratio. Expected fraction of patients missing from the follow-up due to mortality from those born before 2004.

Birth cohort	Before 1975	1976-1980	1981-1985	1986-1990	1991-1995	1996-2000	2001-2005	2006-2010	2011-2015
Number of CF patients	479	330	485	627	626	682	739	628	249
Live births (France, Millions)		3.759	3.879	3.845	3.654	3.718	3.836	3.974	3.906
Fraction of live births in France (/10,000)	-	0.9	1.3	1.6	1.7	1.8	1.9	1.6	0.6
Average case scenario : 1.75 CF for 10,000 births									
Expected number of live CF births*		658	679	673	639	651	671	695	684
Fraction missing			29%	7%	2%	-	-	-	-
Average Mortality**			12%	6%	3%	1%	0.5%	-	-
Worst case scenario : 1.9 CF for 10,000 births									
Expected number of live CF births*		714	737	731	694	706	729	755	742
Fraction missing			34%	14%	10%	3%	-	-	-
Average Mortality**			12%	6%	3%	1%	0.5%	-	-

*Computed as live births × CF rate at birth; **Applying death rates from the 1992-1996 cohort.

Abbreviations: CF: cystic fibrosis

Table S2: Cumulative incidence of items defining CFLD and severe CFLD

Age	CFLD			Severe CFLD		
(years)	Clinical (%)	Biochemical (%)	Ultrasound (%)	Cirrhosis (%)	Portal Hypertension (%)	Esophageal Varices (%)
5	6	15	2	0.3	0.4	0.2
10	12	22	6	1.3	1.7	0.8
15	18	30	12	3.3	4.2	2.5
20	25	40	18	4.7	5.4	3.1
25	33	52	25	5.8	6.9	3.5
30	35	56	28	6.1	6.9	3.5

Abbreviations: CFLD: cystic fibrosis related liver disease

Table S3: Characteristics of the patients with cystic fibrosis according to birth cohort

	1985-1995 (n=1,180)	1995-2005 (n=1,326)
CFTR F508del homozygous	49%	51%
Meconium ileus	14%	13%
Sex (males)	52%	52%

Abbreviations: CFTR: cystic fibrosis transmembrane conductance regulator

Table S4: Biochemical characteristics of the patients in the year before CFLD and in the year before severe CFLD diagnosis.

	Year before CFLD	Year before severe CFLD
ASAT (IU/L)	38.5 ± 29.7	44.3 ± 21.3
ALAT (IU/L)	38.5 ± 30.3	44.3 ± 26.4
APRI score	0.13 ± 0.11	0.25 ± 0.25
GGT (IU/L)	36.7 ± 57	55.3 ± 60.7
Total Bilirubin (µmol/L)	9.03 ± 7.1	9.31 ± 5.35
Platelet count (x 10^9/L)	322 ± 106	237 ± 100

Abbreviations: CFLD: cystic fibrosis related liver disease; ASAT: aspartate aminotransferase; ALAT: alanine aminotransferases; APRI score: ASAT to platelet ratio index; GGT: gamma-glutamyl transpeptidase

Table S5: Body mass index and forced expiratory volume in 1 second according to cystic fibrosis related liver disease status in a subset of patients matched for age and sex

	No CFLD	**CFLD**	**Severe CFLD**	**P-value**
n	169	169	169	
Age (yrs), mean ± SD	20.3 ± 5.7	20.3 ± 5.7	20.3 ± 5.7	-
Sex (Male), %	60%	60%	60%	-
CF- BMI percentile (%)	51 ± 27	48 ± 29	45 ± 27	0.15
(Z-score)	(0.05 ± 0.90)	(-0.05 ± 1.0)	(-0.14 ± 0.89)	
CF-FEV_1 percentile (%)	54 ± 29	50 ± 28	45 ± 28	0.01
(Z-score)	(0.06 ± 0.93)	(-0.09 ± 0.93)	(-0.21 ± 0.91)	

Abbreviations: CFLD: cystic fibrosis related liver disease; BMI: body mass index; FEV_1: forced expiratory volume in 1 second

Table S6 : Incidences of CFLD, severe CFLD and UDCA prescription in the whole cohort and in the patients born after 2004

Age (Years)	Cumulative incidence of CFLD (%[95%CI]) ALL	Cumulative incidence of CFLD (%[95%CI]) ≥ 2004	Cumulative incidence of severe CFLD (%[95%CI]) ALL	Cumulative incidence of severe CFLD (%[95%CI]) ≥ 2004	Cumulative incidence of UDCA (%[95%CI]) ALL	Cumulative incidence of UDCA (%[95%CI]) ≥ 2004
5	3.7 [3.0, 4.5]	6.0 [4.6,7.6]	0.5 [0.3, 0.8]	0.7 [0.2, 1.2]	10 [8.5, 10.7]	13 [11.0, 15.2]
10	9.9 [8.7, 11.0]	13.1 [10.5, 15.7]	2.4 [1.7, 2.9]	1.7 [0.8, 2.7]	18 [16.6, 19.4]	19.0 [15.8, 21.5]

Supplementary figures

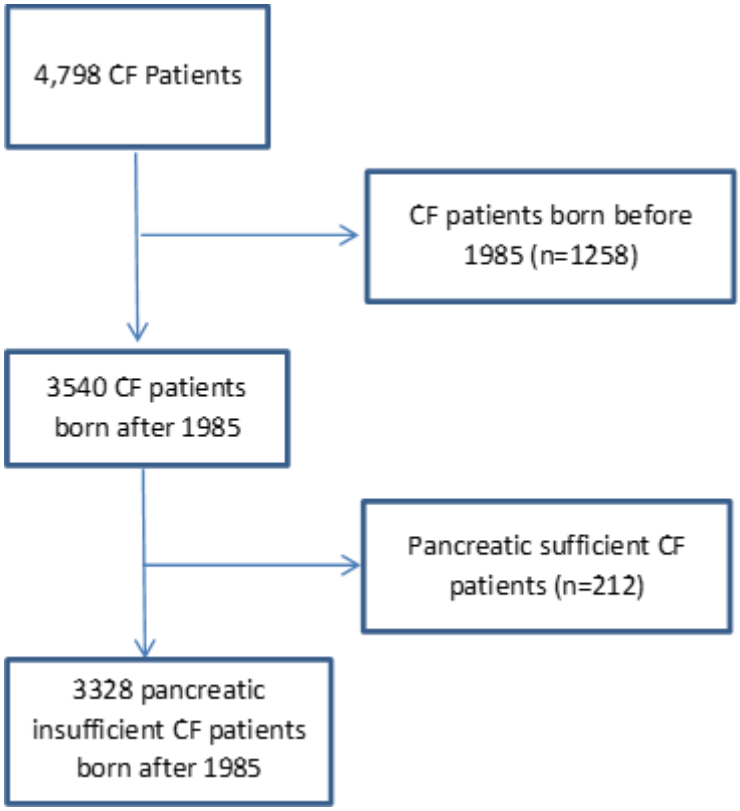

Figure S1: Flowchart of inclusions in the CF liver study

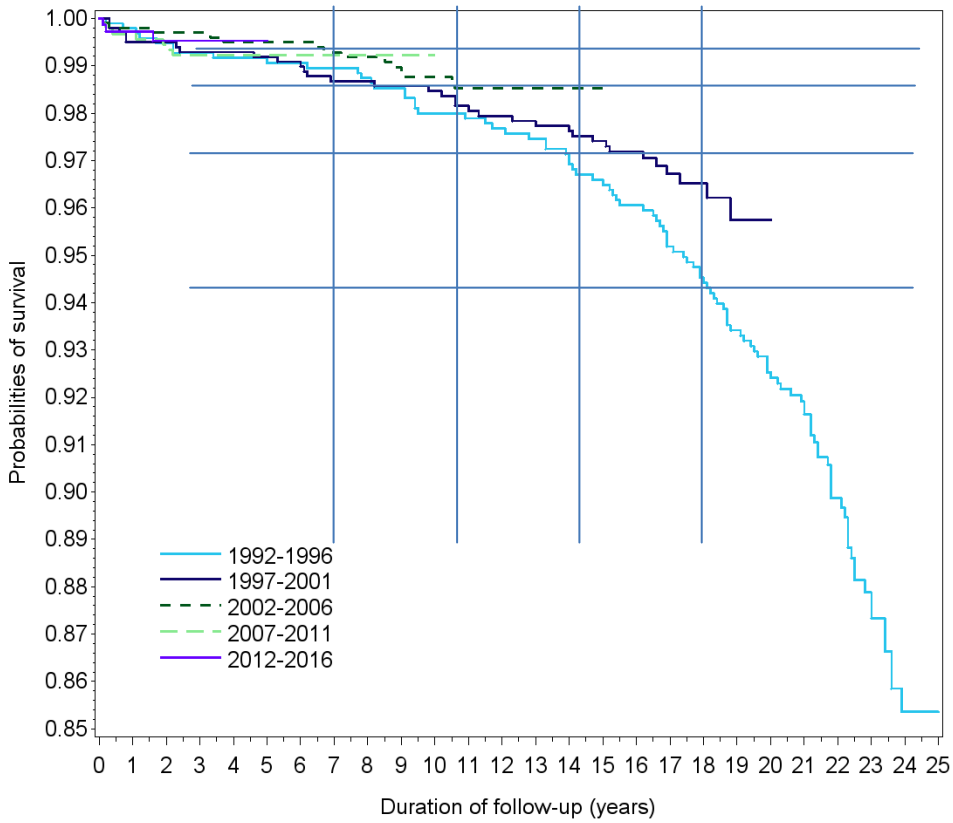

Figure S2: Survival probabilities in French CF patients by birth cohort. *Source*: French CF registry report 2016 (2)

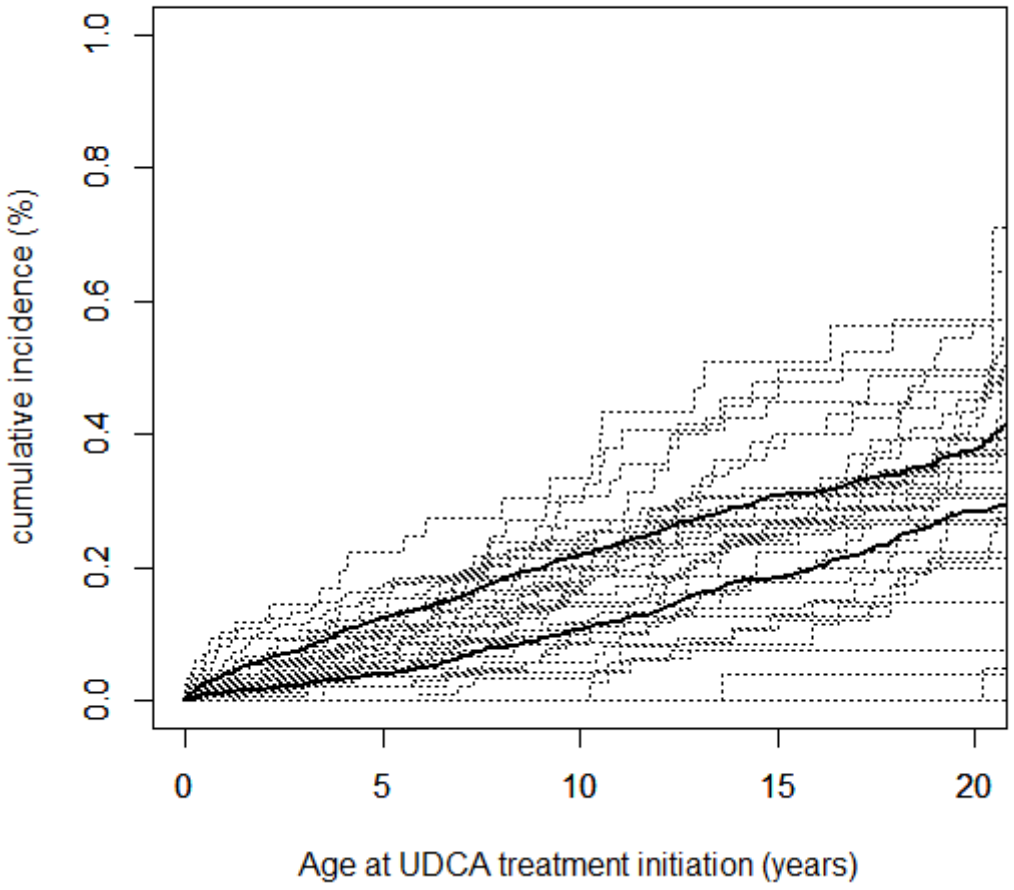

Figure S3: Cumulative incidence of UDCA treatment according to CF center and age. Dashed lines correspond to individual CF centers. Solid lines correspond to the two groups (early and late) based on prescribing rate at age 5.

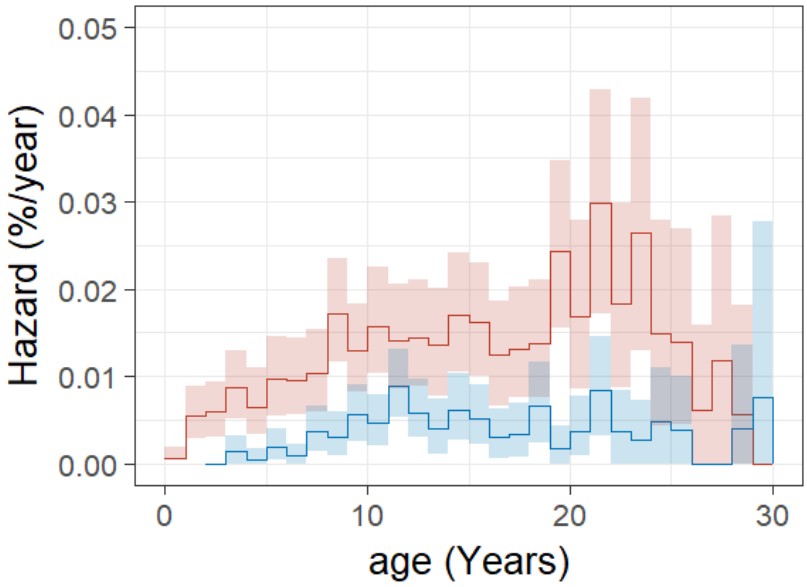

Figure S4: Average annual hazard (%/year) for CFLD (red) and severe CFLD (blue)

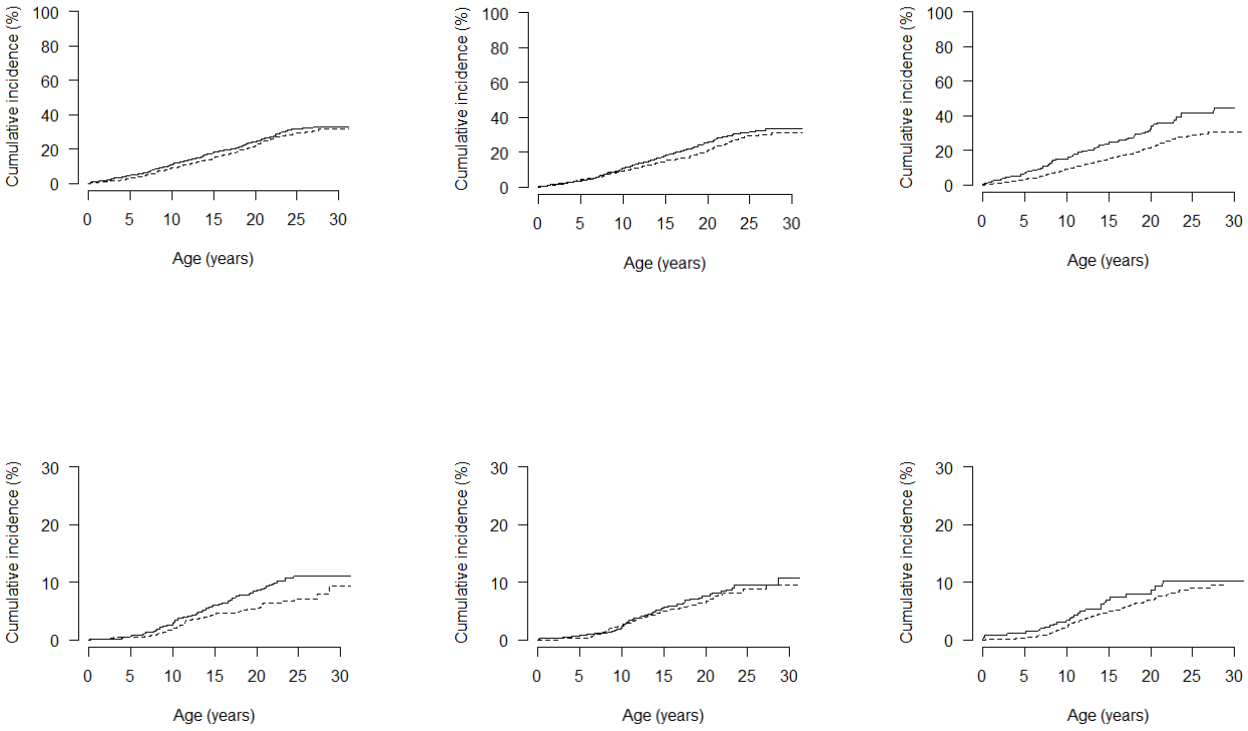

Figure S5: Cumulative incidence of CFLD (top row) and severe CFLD (bottom row) with sex (left column; plain male, dashed female), *CFTR* genotype (middle column; plain F508del homozygosity, dashed other), and meconium ileus (right column; plain history at birth, dashed no history)

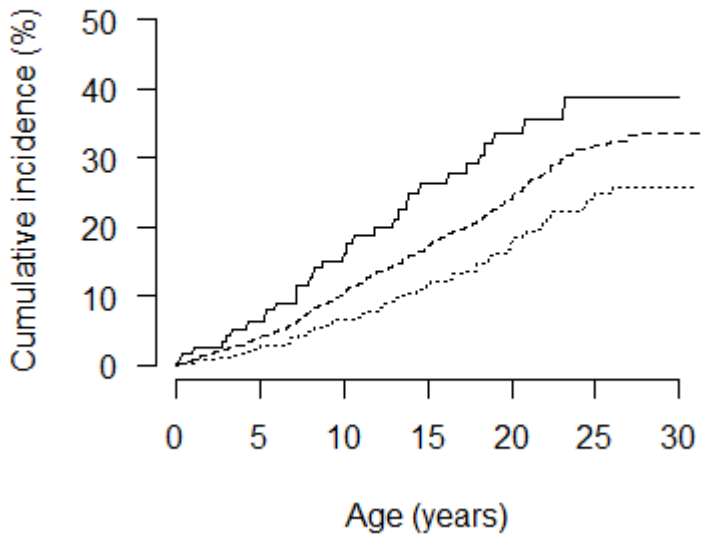

Figure S6: Cumulative incidence of CFLD according to combination of risk factors comparing the group the most at risk (plain: CFTR F508del homozygous males with meconium ileus, 4% of the population), with the group least at risk (dotted: not CFTR F508del homozygous females without meconium ileus, 21% of the population) and all others (dashed, 75% of the population).

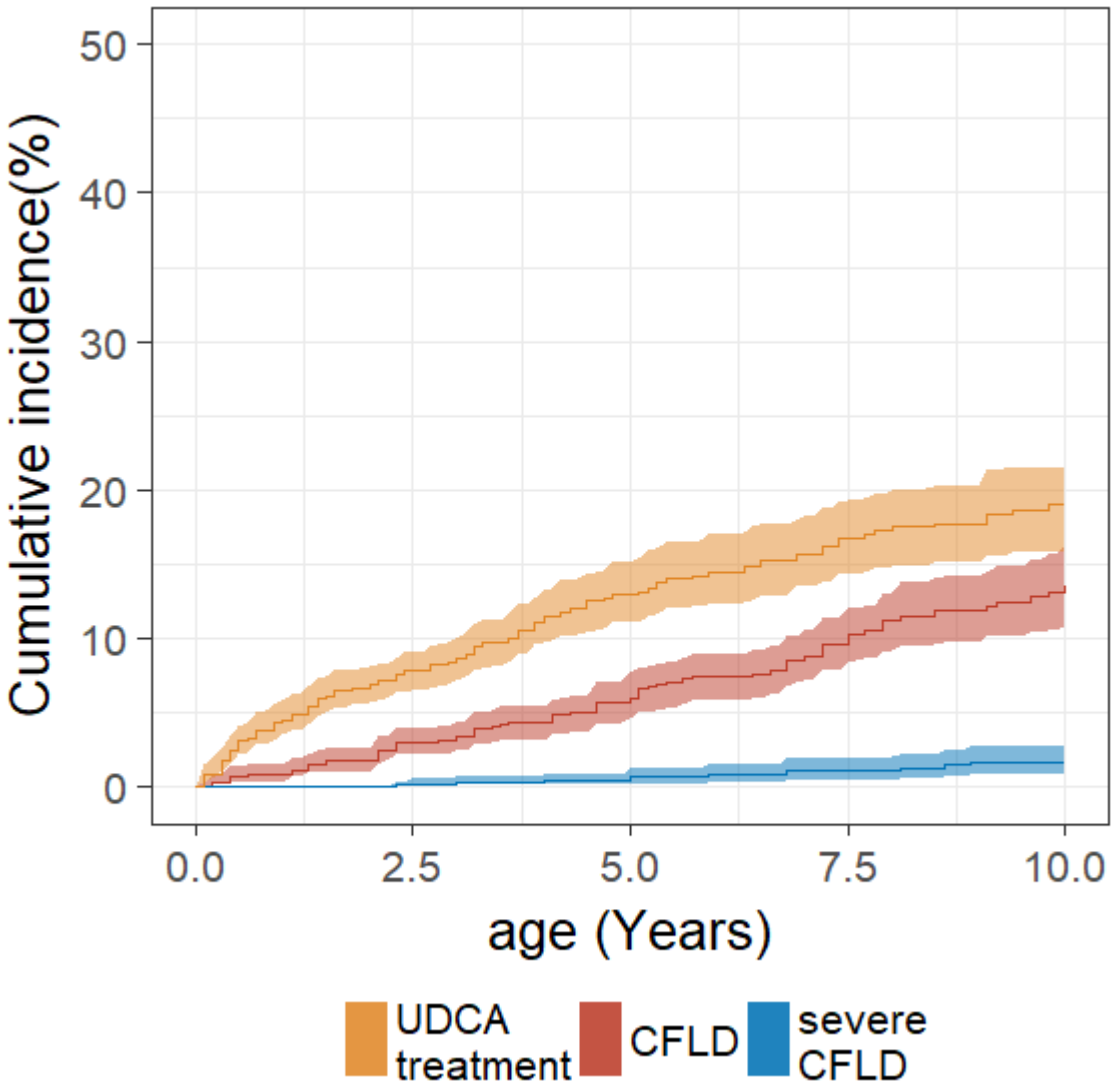

Figure S7: Cumulative incidence of UDCA treatment (orange), CFLD (red), severe CFLD (blue) in CF patients born after 2004.

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