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GENETIC MODIFIERS OF LIVER DISEASE IN CYSTIC FIBROSIS

Jaclyn R. Bartlett, PhD, **Kenneth J. Friedman, PhD**, **Simon C. Ling, MB, ChB**, **Rhonda G. Pace, BS**, **Scott C. Bell, MD**, **Billy Bourke, MD**, **Giuseppe Castaldo, MD**, **Carlo Castellani, MD**, **Marco Cipolli, MD**, **Carla Colombo, MD**, **John L. Colombo, MD**, **Dominique Debray, MD**, **Adriana Fernandez, MD**, **Florence Lacaille, MD**, **Milan Macek Jr., MD, DSc**, **Marion Rowland, MB, PhD**, **Francesco Salvatore, MD**, **Christopher J. Taylor, MD**, **Claire Wainwright, MD**, **Michael Wilschanski, MBBS**, **Dana Zemková, CSc**, **William B. Hannah, BS**, **M. James Phillips, MD**, **Mary Corey, PhD**, **Julian Zielenski, PhD**, **Ruslan Dorfman, PhD**, **Yunfei Wang, MS**, **Fei Zou, PhD**, **Lawrence M. Silverman, PhD**, **Mitchell L. Drumm, PhD**, **Fred A. Wright, PhD**, **Ethan M. Lange, PhD**, **Peter R. Durie, MD**[*], and **Michael R. Knowles, MD**[*] **for the Gene Modifier Study Group**

Cystic Fibrosis/Pulmonary Research and Treatment Center (Drs Bartlett and Knowles, Ms Pace, and Mr Hannah) and Department of Genetics (Dr Lange and Mr Wang), School of Medicine, and Department of Biostatistics, School of Public Health (Drs Zou, Wright, and Lange, and Mr Wang), University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; Center for Molecular Biology and Pathology, Laboratory Corporation of America, Research Triangle Park, North Carolina (Dr Friedman); Division of Gastroenterology, Hepatology and Nutrition (Drs Ling and Durie), Pediatric Laboratory Medicine (Dr Phillips), Child Health Evaluative Sciences (Dr Corey),

Corresponding Author: Michael R. Knowles, MD, Cystic Fibrosis/Pulmonary Research and Treatment Center, The University of North Carolina at Chapel Hill, 7019 Thurston-Bowles Bldg., CB# 7248, Chapel Hill, NC 27599 (knowles@med.unc.edu).
*These authors jointly directed the project.

Author Contributions: Dr Knowles had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Bartlett, Friedman, Ling, Pace, Zou, Silverman, Wright, Lange, Durie, Knowles.
Acquisition of data: Bartlett, Friedman, Ling, Pace, Bell, Bourke, Castaldo, Castellani, Cipolli, C. Colombo, J. Colombo, Debray, Fernandez, Lacaille, Macek, Rowland, Salvatore, Taylor, Wainwright, Wilschanski, Zemkova, Hannah, Phillips, Corey, Zielenski, Dorfman, Silverman, Drumm, Durie, Knowles.
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Critical revision of the manuscript for important intellectual content: Bartlett, Friedman, Ling, Pace, Bell, Bourke, Castaldo, Cipolli, C. Colombo, Debray, Lacaille, Macek, Rowland, Salvatore, Wainwright, Wilschanski, Corey, Zielenski, Dorfman, Drumm, Wright, Lange, Durie, Knowles.
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Abstract

Context—A subset (~3–5%) of patients with cystic fibrosis (CF) develops severe liver disease (CFLD) with portal hypertension.

Objective—To assess whether any of 9 polymorphisms in 5 candidate genes (*SERPINA1, ACE, GSTP1, MBL2*, and *TGFB1*) are associated with severe liver disease in CF patients.

Design, Setting, and Participants—A 2-stage design was used in this case–control study. CFLD subjects were enrolled from 63 U.S., 32 Canadian, and 18 CF centers outside of North America, with the University of North Carolina at Chapel Hill (UNC) as the coordinating site. In the initial study, we studied 124 CFLD patients (enrolled 1/1999–12/2004) and 843 CF controls (patients without CFLD) by genotyping 9 polymorphisms in 5 genes previously implicated as modifiers of liver disease in CF. In the second stage, the *SERPINA1* Z allele and *TGFB1* codon 10 genotype were tested in an additional 136 CFLD patients (enrolled 1/2005–2/2007) and 1088 CF controls.

Main Outcome Measures—We compared differences in distribution of genotypes in CF patients with severe liver disease versus CF patients without CFLD.

Results—The initial study showed CFLD to be associated with the *SERPINA1* (also known as α1-antiprotease and α1-antitrypsin) Z allele (P value=3.3×10^{-6}; odds ratio (OR) 4.72, 95% confidence interval (CI) 2.31–9.61), and with transforming growth factor β-1 (*TGFB1*) codon 10 CC genotype (P=2.8×10^{-3}; OR 1.53, CI 1.16–2.03). In the replication study, CFLD was associated with the *SERPINA1* Z allele (P=1.4×10^{-3}; OR 3.42, CI 1.54–7.59), but not with *TGFB1* codon 10. A combined analysis of the initial and replication studies by logistic regression showed CFLD to be associated with *SERPINA1* Z allele (P=1.5×10^{-8}; OR 5.04, CI 2.88–8.83).

Conclusion—The *SERPINA1* Z allele is a risk factor for liver disease in CF. Patients who carry the Z allele are at greater odds (OR ~5) to develop severe liver disease with portal hypertension.

Cystic fibrosis (CF) is a recessive monogenic disorder characterized by multi-organ involvement and clinical heterogeneity that is incompletely explained by mutations within the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene.[1] Patients with CF, including those homozygous for DF508, exhibit a range of lung disease severity, and genetic variability in non-*CFTR* genes contributes to risk for severity of pulmonary disease.[2–7]

Intrinsic abnormalities in the CF liver reflect loss of CFTR (Cl^- channel) function on the apical membrane of cholangiocytes.[8,9] This dysfunction is predicted to result in defective (sluggish) bile flow, and is associated with a cholangiocyte-induced inflammatory response with activation and proliferation of hepatic stellate cells, which results in cholangitis and fibrosis in focal portal tracts.[10–13] However, only a small fraction (~3–5%) of CF patients develops severe liver disease characterized by cirrhosis with portal hypertension (CFLD)[1]; thus, non-*CFTR* genetic variability may contribute to risk for severe liver disease.[14–17]

To determine the association between non-*CFTR* genetic polymorphisms and severe liver disease in CF with portal hypertension (CFLD), we studied 9 functional variants in 5 genes previously studied in CF liver disease, including α1-antitrypsin (also known as α1-antiprotease, α1AP, *SERPINA1*)[18], angiotensin-converting enzyme (*ACE*)[19], glutathione S-transferase (*GSTP1*)[20], mannose-binding lectin 2 (*MBL2*)[21] and transforming growth factor β1 (*TGFB1*).[19] Our initial study compared polymorphic genotypes in these candidate modifier genes in CF subjects with CFLD and "control" CF patients (without CFLD, who were at least 15 years of age). We tested our initial findings in a second study in different populations of CF patients with and without CFLD.

METHODS

Patients - Initial (First) Study

Of the 158 CF patients evaluated for severe liver disease (CFLD; portal hypertension; enrolled 1/1999–12/2004), there were 128 patients who fulfilled criteria from 22 CF Centers in 10 countries (Australia, 8; Canada, 17; Czech Republic, 17; Germany, 3; Italy, 28; Netherlands, 1; Scotland, 2; Slovakia, 4; Turkey, 4; and U.S., 44). For patients without 2 defined mutations in CFTR, we tested further, using a panel of 70 mutations (Tm Bioscience/Luminex CFTR mutation detection assay). After genotyping was complete, >95% of CFLD patients with 2 defined mutations in *CFTR* had 2 pancreatic insufficient (PI) mutations (eTable 1). The 843 control patients (CF patients without CLFD) were enrolled from the U.S. (759 patients from 42 Centers) and Canada (84 patients from 32 Centers). The majority of the CF controls were ascertained from the GMS Lung Study population (DF508 homozygotes; 92.6%)[5], Most of the other controls had biallelic PI mutations (see online supplement, Methods). These controls without CFLD were ≥ 15 years of age (1 SD above the mean age of diagnosis of CFLD), in order to exclude younger patients who might have occult liver disease.

Patients - Replication (Second) Study

Of the 191 CF patients evaluated for CFLD (portal hypertension; enrolled 1/2005–2/2007), there were 139 CF patients who fulfilled criteria from 35 CF Centers in 10 countries (Argentina, 5; Australia, 5; Canada, 24; Chile, 1; France, 9; Ireland, 8; Israel, 7; Italy, 14; United Kingdom, 4; and U.S., 62). The percentage of PI *CFTR* genotypes in CFLD subjects was similar to those in the initial study (eTable 1). The 1088 control patients (CF patients ≥ 15 years without CFLD) were ascertained from five countries (Canada, 391 patients from 32 Centers; Czech Republic, 30 patients; Ireland, 6 patients; Italy, 71 patients; and U.S., 590 patients from 54 Centers). The majority of the CF controls had 2 PI mutations (93.5%; mostly DF508/DF508 62.8%) (online supplement, Methods).

Enrollment Criteria

All patients had a diagnosis of CF, confirmed by sweat test and/or *CFTR* genotyping. CFLD was defined as cirrhosis in patients ≥ 2 years of age, confirmed by imaging (ultrasound, CT, MRI) showing hepatic parenchymal abnormalities of portal hypertension (esophageal varices, portal-systemic collaterals, splenomegaly) in the absence of another cause for liver disease. Data were independently reviewed by 2 hepatologists (P.R.D. and S.C.L.) with experience in CFLD to ensure inclusion and exclusion criteria were met, using case report forms, radiology and endoscopy reports, and clinical notes. When there was no consensus, the reviewers requested additional information to clarify the diagnosis of CFLD. No patient was excluded because of race or ethnic background, which were subject-defined.

We excluded 30 (19%) and 52 (27%) subjects originally submitted for the initial and replication studies, respectively, with a presumed diagnosis of CFLD, because they had milder liver disease without portal hypertension, or inadequate documentation. For the 47 patients with confirmed CFLD who had a liver transplant (26 in initial study; 21 in replication study), source documents were obtained from dates prior to transplant. Exclusion criteria for the CFLD group included portal vein thrombosis or other causes of liver disease (alcohol abuse, biliary atresia, clinically significant viral hepatitis, use of parenteral nutrition, and Wilson disease). The study was approved by the Institutional Review Boards of all participating institutions and written informed consent was obtained.

Exclusion from Analysis Based on Age of Diagnosis of CFLD

In common with previous reports, we found the mean age of diagnosis of CFLD (first documentation of portal hypertension) to be 10.6 (± 5.4) years (eFigure 1).[15,22–25] The diagnosis of CFLD was first established after the age of 30 years in 7 subjects (ages of 32, 33, 35, 40, 43, 44, and 47 years), which is ≥ 4 SD above the mean of the normal distribution. Therefore, these patients were excluded from the genetic analyses (4 from the initial study, and 3 from the replication study).

Data Collection

Patients received a unique identifier code, and data was stored in a secure database in the UNC Bioinformatics Center. Clinical data on standard case report forms included self-reported race/ethnicity, pancreatic exocrine status, medical history, physical examination, laboratory blood work values, and abdominal radiology reports. In addition, we reviewed the following procedure reports if available: liver explant pathology (from liver transplant), liver biopsy, endoscopy and colonoscopy.

DNA Extraction and Genotyping

DNA was extracted from peripheral blood leukocytes using standard protocols.[26] Genetic polymorphisms were determined by direct sequencing, by microsphere-based genotyping using Illumina BeadArray™ technology (San Diego, CA) and by site-directed mutagenesis (online supplement, Methods).

Histochemistry/Histopathology

Immunohistochemistry with polyclonal rabbit anti-α-1 antitrypsin antibody and monoclonal mouse anti-CD68 (clone KP1) antibody (Dako Canada, Ontario, Canada), was performed on the Benchmark XT™ auto-immunostainer (Ventana Medical Systems, Tucson, AZ) at dilutions of 1:3000 and 1:5000, respectively. Immunodetection was performed using the Ventana, i-VIEW DAB, LSAB kit. Tissue sections were dewaxed, enzyme pretreated for α-1 antitrypsin, heat epitope retrieved for CD68, peroxidase, and endogenous biotin blocked using Ventana proprietary reagents. Sections were hematoxylin counterstained for nuclear detail (eFigure 2).

Statistical Analysis

Genotype distributions were tested for consistency with expected Hardy-Weinberg equilibrium (HWE) proportions for cases and controls in the initial, replication and combined studies, using all subjects and then restricted to Caucasian subjects, using the PLINK software package (version 1.03; http://pngu.mgh.harvard.edu/~purcell/plink/).[27]

For the initial study, the association between polymorphisms and CFLD was assessed using Cochran-Armitage trend tests.[28] All tests were 2-sided and unadjusted P values are reported, along with P values that were significant ($P<0.05$) after Bonferroni (adjusted for 9 tests) correction (appended to Tables). Analyses were performed using all samples, and only Caucasian samples.

For the replication study, the association between 2 polymorphisms from the initial study (*SERPINA1* Z allele, and *TGFB1* codon 10) and CFLD was assessed using Cochran-Armitage trend tests. Initial and replication samples were subsequently combined and analyzed for the *SERPINA*1 Z allele using Cochran-Armitage trend tests and logistic regression models (online supplement, Methods). Varying levels of covariate adjustment in the logistic regression models were made for ethnicity (as a 5-level categorical variable for all samples), gender, *CFTR* genotype and *TGFB1* codon 10 genotype. Tests of interactions were performed to assess whether the odds of CFLD differed between males and females by *SERPINA1* genotype. Odds ratios (and corresponding 95% confidence intervals) and uncorrected P values are reported. Bonferroni correction was applied to assess overall statistical significance in the replication and combined analyses (adjusting for 2 tests in the replication and 9 tests in the combined sample). Analyses were performed separately, using all samples and then using Caucasian subjects only.

Analysis of variance models were used to assess whether gender, *SERPINA1* Z allele and *CFTR* genotype were associated with age of diagnosis of CFLD in the combined sample. Data were analyzed on all CFLD cases and Caucasian CFLD cases with a reported age at diagnosis with covariate adjustment for self-reported ancestry.

To estimate population attributable risk, we used a modified form of Levin's classic formula for population attributable fraction by replacing relative risk estimates with odd ratios, and using the proportion of control subjects carrying the Z allele as an estimate of the probability of exposure[29–31] (online supplement, Methods). While this estimate is not exact, given our case–control sampling design (over-sampled older CF patients without CFLD), this estimate should provide a reasonable approximation, due to the modest frequency of CFLD in CF patients (~5%).

RESULTS

Clinical Features - Initial Study

Characteristics of the initial group of 124 CF patients with severe liver disease and 843 CF patients without CFLD are shown in Table 1. The CFLD group was younger at enrollment, had more males, and slightly fewer Caucasians. The *CFTR* mutations in CFLD patients were representative of PI mutations in North American and European CF patients (Table 1, eTable 1)[1]. The prevalence of meconium ileus at birth in CFLD patients (18.2%) is comparable to the control (no CFLD) group, and typical for the general CF population with pancreatic insufficient *CFTR* mutations.[1]

Abnormalities in biochemical tests of the liver (AST, ALT and GGT) were not predictive of CFLD and showed no correlation with markers of hepatocellular synthetic dysfunction, such as international normalized ratio (INR) and serum albumin (Table 2). Preoperative assessment of data available from a subset of patients (n=22) who underwent liver transplant (n=43) showed a similar distribution of abnormal total bilirubin and, albumin values as the non-transplanted patients (Table 2, footnote "b" and "c").

Cochran-Armitage Trend Test of Association – Initial Study

In the analysis of previously studied gene modifiers of liver disease in CF (Table 3), association was seen only for the *SERPINA1* Z allele ($P=3.3\times10^{-6}$; OR 4.72, CI 2.31–9.61) and *TGFB1* codon 10 ($P=2.8\times10^{-3}$; OR 1.53, CI 1.16–2.03). The *SERPINA1* Z allele displayed association for all CFLD patients, but was more prominent in females (eTable 2). Similar results were seen when the analysis was restricted to Caucasians (data not shown). It is noteworthy that small effects for the non-significant polymorphisms would not be detected with sufficient power by this study (see online supplement Methods – Power Analysis; eTable 3). The genotypes and minor allele frequencies for genetic variants in patients without CFLD were similar to those previously reported.[5–7,18–21]

Clinical Features - Replication Study

Based on the associations for the *SERPINA1* Z allele and *TGFB1* codon 10 (Table 3), we enrolled additional CF patients with and without CFLD to test for replication (Table 4). The characteristics of the replication patients were similar to those in the initial study (Table 1), including the distribution of specific CFTR mutations (eTable 1), prevalence of meconium ileus (23.8%) and liver function abnormalities (Table 2, Table 4).

Cochran-Armitage Trend Test of Association – Replication Study

The association was replicated for the *SERPINA1* Z allele ($P=1.4\times10^{-3}$; OR 3.42, CI 1.54–7.59); Table 5), but the association was more prominent in males (eTable4), in contrast to the initial study (eTable 2). Similar results were seen when analyses were restricted to Caucasians (data not shown). The association of the *TGFB1* codon 10 variant was not replicated for all patients (Table 5) or for males or females when analyzed separately (data not shown).

Cochran-Armitage Trend Test of Association – Initial plus Replication Study

When the initial and replication populations were combined for analysis using Cochran-Armitage trend tests, the *SERPINA1* Z allele displayed very robust association with CFLD ($P=9.9\times10^{-9}$; OR 4.17, CI 2.46–7.05 eTable 4); similar evidence for association was observed in analyses restricted to Caucasian subjects in the initial plus replication populations(data not shown).

Hardy-Weinberg Equilibrium

All polymorphisms had genotype distributions consistent with Hardy-Weinberg equilibrium ($P > 0.01$) in the initial, replication, and combined samples, irrespective of how samples were partitioned according to ethnicity and CFLD status.

Logistic Regression for the Z Allele – Initial plus Replication Study

We combined the initial and replication groups and performed logistic regression for the *SERPINA1* Z allele to estimate the odds of CFLD, adjusting for the covariates of ethnicity, gender and *CFTR* genotype. Results remained consistent when using all subjects or Caucasian subjects only, with respect to both statistical significance estimates ($P=1.5\times10^{-8}$ or $P=6.3\times10^{-8}$, respectively) and odds ratio estimates (OR 5.04, CI 2.88–8.83 for all patients vs. OR 4.87, CI 2.75–8.64 for Caucasians only). In addition, we saw no evidence for interactions between gender and the *SERPINA1* Z allele in all subjects or only Caucasians. Similar results were obtained by logistic regression adjusting only for ethnicity in the complete sample, and models that additionally adjusted for the *TGFB1* codon 10 genotype (eTable 5).

Population Attributable Risk

We combined the initial and replication groups, and the population attributable risk for the Z allele was estimated to be 6.7% (Caucasians only, 6.6%) (see online supplement, Methods). A similar result was obtained using another method of estimating the probability of exposure, namely the average Z allele frequency of patients from North America, Europe and Australia (data not shown).

Age of Diagnosis of CFLD

The mean and median age of recognition (diagnosis) of portal hypertension in all CFLD patients was ~10–11 years (eFigure 1) and 90% of patients had CFLD diagnosed before 20 years of age. Males had an earlier age of diagnosis of CFLD than females for all subjects (males=8.5 yrs, females=10.5 years; P=0.007), and for self-reported Caucasians (males=9.7 years, females=11.5 years; P=0.027). Age at diagnosis of CFLD was not associated with the presence of the *SERPINA1* Z allele, *CFTR* genotype or self-reported ancestry.

Liver Histopathology

A CFLD subject carrying a single copy of the *SERPINA1* Z allele accumulated SERPINA1 protein within hepatocytes adjoining the fibrosed portal tracts, but SERPINA1 protein is not seen in hepatocytes of a CFLD patient without the Z allele (eFigure 2).

COMMENT

Previous studies have suggested that genetic polymorphisms may act as modifiers of liver disease in cystic fibrosis, but these studies were small and phenotyping did not address the development of severe (biliary) cirrhosis associated with portal hypertension.[18–21] To increase the likelihood of identifying genetic modifiers that are relevant to the development of severe liver disease in CF, i.e., cirrhosis with portal hypertension, we performed 2 sequential studies in different groups of patients. The initial study involved 5 candidate genes that had previously been studied as modifiers of CF liver disease[18–21], and the replication study tested for confirmation of *SERPINA1* Z allele and *TGFB1* codon 10 variant as modifiers of severe liver disease in CF.

This study had 3 key design features. First, we used rigorous criteria to identify CF patients with portal hypertension (“cases”), reflecting hepato-biliary cirrhosis, and key source documents were reviewed independently by 2 experts to confirm the CFLD phenotype. Second, for CF patients without CFLD (“controls”), we studied only patients who were ≥ 15 years of age, in order to exclude younger patients with predisposition to develop CFLD. Third, we enrolled a large number of CF patients with and without CFLD in order to improve statistical power. For the initial study, ~50% of the CFLD patients were from outside North America and 93% were self-described as Caucasian, and all control CF patients were from North America. For the replication (second) study, a slightly greater percentage of CFLD patients were from North America (63% versus 50%), and there were some control CF patients (10%) from outside North America.

Genetic analyses of the initial cohort showed that a single copy of the *SERPINA1* Z allele and each additional copy of the *TGFB1* codon 10 C allele were associated with significantly increased odds of CFLD. In the replication study, the *SERPINA1* Z allele was confirmed as a modifier of liver disease in CF, whereas the *TGFB1* codon 10 variant was not confirmed. It is noteworthy that small effects of the non-significant polymorphisms in other genes would not be detected with sufficient power by this study. The association of the *SERPINA1* Z allele with CFLD contrasts to a previous "negative" study, which used less stringent phenotypic markers of CF liver disease, such as liver function tests, which do not correlate with severity of CFLD (portal hypertension).[18]

When the initial and replication study populations were combined for joint analysis by multivariable logistic regression, the magnitude of the effect of the *SERPINA1* Z allele was large compared to most genetic association studies (odds ratio ~5) when gender, ethnicity and *CFTR* genotype were included as covariates. The strength of the association of the *SERPINA1* Z allele with CFLD varied by gender for the initial versus the replication studies, but the overall odds were not statistically different for females and males when all subjects were analyzed. Population stratification is unlikely to account for the results for the *SERPINA1* Z allele; the prevalence of the Z allele (1.14%) in our CF patients without CFLD (controls) is similar to that reported for > 85,000 individuals genotyped in pertinent regions of the world (1.20%).[32,33] (eFigure 3 and eTable 4).

The mechanism of the *SERPINA1* Z allele as an adverse modifier of liver disease in CF patients likely reflects the dual stimulation of hepatic stellate cells by inflammatory mediators from both *CFTR*-deficient cholangiocytes and hepatocytes containing the misfolded SERPINA1 protein, i.e., these inflammatory stimuli induce hepatic stellate cells to migrate and proliferate in the bile duct regions in a pro-fibrogenic manner.[10–13,16,34–38] Bile duct ligation with resultant cholestasis induces more activated stellate cells and fibrosis in the liver of homozygous transgenic PiZ versus wild-type mice, which is compatible with this proposed mechanism of the Z allele as an adverse modifier in CF.[39] Further studies are necessary to better define the pathogenesis of the Z allele in CFLD.

The Z allele variant causes misfolding of the SERPINA1 protein, which results in an accumulation of protein in hepatocytes. The most prevalent *CFTR* mutation, DF508, is also a misfolding mutation, expressed predominantly in cholangiocytes in the liver.[8–13,34–38] However, it is unlikely that folding mutations in CFTR and SERPINA1 induce an amplified, adverse effect on the proteosomal degradation pathway, because these 2 genes are predominantly expressed in 2 different cell types in the liver.[8,9,34–38] Furthermore, heterozygosity for the Z allele is associated with the risk and progression of a variety of liver diseases, including cryptogenic cirrhosis, biliary atresia, viral hepatitis, alcoholic cirrhosis and non-alcoholic fatty liver disease.[37,38,40]

By studying a large number of CF patients with well-defined severe liver disease and portal hypertension, we confirmed and refuted some previous observations and discovered new information about the clinical features of these patients. We confirmed that 1) CFLD is more common, and diagnosed earlier, in males; 2) specific *CFTR* mutations do not correlate with CFLD, but *CFTR* mutations with residual function (pancreatic sufficient mutations) are uncommon in subjects with CFLD; 3) hepatic synthetic function is preserved for long durations in most CFLD patients; 4) thrombocytopenia due to hypersplenism is common in subjects with portal hypertension due to CFLD; 5) liver biochemical tests are poorly predictive of severe liver disease and portal hypertension in CF and 6) severe liver disease (portal hypertension) develops in pediatric patients by age 10–12 years.[14–17,22–25] In addition, we made a striking observation about the age distribution of diagnosis of severe liver disease, whereby the prevalence of severe liver disease does not increase in CF adults, despite progressive increase in longevity; more than 90% of our CFLD patients were diagnosed by age 20, with a mean (and median) age of diagnosis of 10–11 years. We were not able to confirm any association of meconium ileus with CFLD, as previously reported in some, but not all, studies;[15–17,22–25,41] the prevalence of meconium ileus in our patients with CFLD (~20%) was similar to that reported for pancreatic exocrine insufficient patients.[1]

In summary, we studied 2 large populations of CF patients with and without liver disease and portal hypertension in order to test genes previously studied as modifiers of liver disease. Of these candidate genes, only the *SERPINA1* Z allele was significantly associated with CFLD and portal hypertension. This polymorphism is relatively uncommon in CF (~2.2% of CF patients are carriers), but the odds ratio for association with severe liver disease is relatively high (~5) for the contribution of a genetic modifier to a Mendelian disorder. Moreover, the estimated population attributable risk among CF patients is 6.7%. From a clinical perspective, a rare variant with large penetrance (such as the Z allele) may be more useful than a common variant with low penetrance, to screen for genetic polymorphisms. The availability of this first marker, *SERPINA1* Z allele, for the development of severe liver disease in CF illustrates the possibility of identifying risk factors in CF patients early in life, conceptually as a secondary component of neonatal screening after the diagnosis of CF is confirmed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Participating Groups

United States of America

Alabama: University of Alabama at Birmingham, Birmingham -- J.P. Clancy; Pulmonary Associates, Mobile -- L.J. Sindel.

Alaska: Providence Alaska Medical Center, Anchorage -- D.M. Roberts and V. Roberts.

Arizona: Phoenix Children’s Hospital, Phoenix -- P.J. Radford and N. Argel; University of Arizona, Tucson -- W.J. Morgan and J.L. Douthit.

Arkansas: University of Arkansas Medical Sciences, Little Rock -- D.E. Schellhase, P. Anderson and A. Taggart.

California: University of California, Davis School of Medicine, Davis -- B. Morrissey; Children’s Hospital of LA, Los Angeles -- A.C.G. Platzker, M.S. Woo, L. Fukushima and E. Hsu; Northern California Kaiser Permanente Medical Care Program, Oakland -- G.F. Shay;

Bay Area Pediatric Pulmonary, Oakland -- K.A. Hardy; Lucile Packard Children's Hospital, Stanford University Medical Center, Palo Alto -- R.B. Moss and C.E. Dunn; University of California-San Diego, Children's Hospital, San Diego -- M.S. Pian; Naval Medical Center, San Diego -- H.A. Wojtczak and L. Burns; California Pacific Medical Center, San Francisco -- N.R. Henig; University of California San Francisco, San Francisco -- D.W. Nielson; Ventura County Medical Center, Ventura -- C. Landon and A. Thompson.

Colorado: Denver Children's Hospital, Denver -- F.J. Accurso; University of Colorado Adult Cystic Fibrosis Program, Denver -- J.A. Nick and M. Jones.

Connecticut: Connecticut Children's Medical Center, Hartford -- C. Lapin and V.M. Drapeau; Cystic Fibrosis Care Center, Yale University, New Haven -- M.E. Egan.

Delaware: Nemours/Alfred I. Dupont Hospital for Children, Wilmington -- R. Padman.

Washington, D.C.

Children's National Medical Center, Washington -- G.B. Winnie and C. George.

Florida: University of Florida, Gainesville -- E.L. Olson; Batchelor Children's Institute, University of Miami, Miami -- M.J. Light; Nemours Children's Clinic - Orlando, Orlando -- D.E. Geller; All Children's Hospital, St. Petersburg -- M. Gondor and J. Flanary.

Georgia: Emory University CF Center, Atlanta -- A.A. Stecenko; Medical College of Georgia, Augusta -- M.F. Guill.

Illinois: Northwestern University Feinberg School of Medicine, Chicago -- S.A. McColley; Children's Memorial Hospital, Chicago -- S.A. McColley and E.M. Potter; Loyola University Medical Center, Maywood -- Y. Chung and M. Garvey.

Indiana: Riley Hospital for Children, Indianapolis -- M.S. Howenstine; Indiana University School of Medicine - Adult Cystic Fibrosis Center, Indianapolis -- A. Sannuti and J. Yeley.

Iowa: Blank Children's Hospital, Des Moines -- D.G. Sloven; University of Iowa, Iowa City -- R.C. Ahrens and M. Teresi.

Kansas: PriVia, The Research Centers of Via-Christi, Wichita -- C.M. Riva.

Louisiana: Tulane University School of Medicine, New Orleans -- S. Davis, B. Quiniones-Ellis and C. Gabor.

Maine: Eastern Maine Medical Center, Bangor -- T.F. Lever and R. Welch; Maine Pediatric Specialty Group, Portland -- A. Cairns and M. Corrigan.

Maryland: Johns Hopkins University School of Medicine, Baltimore -- P.L. Zeitlin and L. Brass.

Massachusetts: Massachusetts General Hospital, Boston -- H. Dorkin; Children's Hospital, Boston -- H. Levy and I. Huntington; University of Massachusetts Memorial Health Care, Worcester -- B.P. O'Sullivan.

Michigan: University of Michigan Health System, Ann Arbor -- R.H. Simon, S.Z. Nasr, C.N. Lumeng and M.E. Ball; Children's Hospital of Michigan – Wayne State, Detroit -- D.S. Toder; Michigan State University CF Center, East Lansing -- R.E. Honicky; Spectrum Health Hospitals, Grand Rapids -- S. Fitch and L. Contreras.

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Minnesota: University of Minnesota, Minneapolis -- W.E. Regelmann, J.R. Phillips; Children's Hospitals and Clinics of Minnesota, Minneapolis -- J. McNamara and M. Johnson.

Mississippi: University of Mississippi Medical Center, Jackson -- F.E. Ruiz and K.G. Adcock.

Missouri: University of Missouri, Columbia -- P. Konig; Children's Mercy Hospital, Kansas City -- P. Black and J.D. Weigel; Saint Louis University, St. Louis -- B.E. Noyes and V.L. Kociela; Washington University School of Medicine, St. Louis -- T. Ferkol, Jr. and M. Boyle.

Nebraska: University of Nebraska Medical Center, Omaha -- J.L. Colombo.

Nevada: University of Nevada, Cystic Fibrosis Center, Las Vegas -- T. Brascia.

New Hampshire: Dartmouth Hitchcock Medical Center, Lebanon -- H.W. Parker.

New Jersey: Monmouth Medical Center, Long Branch -- R.L. Zanni; Atlantic Health/ Morristown Memorial Hospital, Morristown -- S.B. Fiel and P. Lomas.

New Mexico: University of New Mexico, Albuquerque -- J. Taylor-Cousar.

New York: The Women and Children's Hospital of Buffalo, Buffalo -- D. Borowitz; Schneider Children's Hospital, New Hyde Park -- J.K. DeCelie-Germana; Long Island Jewish Medical Center, New Hyde Park -- R. Cohen and M. Gannon; Columbia University, New York -- E.A. DiMango; Columbia University Medical Center, New York -- A.A. Mencin, S.J. Lobritto and M. Benitez; St. Vincent’s Hospital, New York -- P.A. Walker, M.N. Berdella and E. Langfelder-Schwind; University of Rochester, Rochester -- C.L. Ren and A.K. Rovitelli; Upstate Medical University, SUNY, Syracuse -- R.D. Anbar and D.M. Lindner; Samaritan Medical Center, Watertown -- R.G. Perciaccante; Maria Fareri Children's Hospital at Westchester Medical Center, Westchester -- A.J. Dozor.

North Carolina: University of North Carolina at Chapel Hill, Chapel Hill -- M.R. Knowles and M.W. Leigh; Duke University, Durham -- J. Taylor-Cousar, J.A. Voynow and K.J. Auten; Wake Forest University Baptist Medical Center, Winston-Salem -- M.S. Schechter.

Ohio: Akron Children's Hospital, Akron -- G.J. Omlor and D.A. Ouellette; Cincinnati Children’s Hospital, Cincinnati -- C.L. Karp; University of Cincinnati Medical Center, Cincinnati -- P.M. Joseph; Rainbow Babies and Children's Hospital, CWRU, Cleveland -- M.W. Konstan; Nationwide Children's Hospital, Columbus -- K.S. McCoy; Children’s Medical Center, Dayton -- F. Royce and S. Bartosik; Toledo Children’s Hospital, Toledo -- P.A. Vauthy and M.L. Vauthy.

Oklahoma: Tulsa Cystic Fibrosis Center, Tulsa -- J.C. Kramer and S. Hensel.

Pennsylvania: Geisinger Health System, Danville -- C.R. Perez; Penn State Children's Hospital and Penn State University College of Medicine, Hershey -- N.J. Thomas and J.C. Hess; Penn Presbyterian Medical Center, Philadelphia -- D.S. Holsclaw; Children's Hospital of Philadelphia-University of Pennsylvania, Philadelphia -- T.F. Scanlin, R. Rubenstein, C. Murray and M. Skotleski; Drexel University College of Medicine, Philadelphia -- S.B. Fiel, W.P. Sexauer, A. Ko and J. Hillman; University of Pittsburgh, Pittsburgh -- D.M. Orenstein.

Rhode Island: Rhode Island Hospital, Providence -- M.S. Schechter.

South Carolina: Medical University of South Carolina, Charleston -- P.A. Flume; University of South Carolina School of Medicine, Columbia -- D. Brown.

Tennessee: University of Tennessee Health Science Center, Memphis -- R. Schoumacher and B. Culbreath; Vanderbilt University Medical Center, Nashville -- P.E. Moore and B. Slovis.

Texas: Cook Children's Health Care System, Fort Worth -- N. Dambro and J. Garbarz; Texas Children's Hospital - Baylor, Houston -- P.W. Hiatt; Wilford Hall Medical Center, San Antonio -- K.N. Olivier; University of Texas Health Science Center, Tyler -- R. Amaro and L. Macleod.

Utah: University of Utah Pulmonary Division, Salt Lake City -- T.G. Liou.

Virginia: University of Virginia Medical Center, Charlottesville -- D.K. Froh; Children's Hospital of The King's Daughters, Norfolk -- C.E. Epstein; Virginia Commonwealth University, Richmond -- J. Schmidt, G. Elliot, R. Williams, M. Anderson and J. Gadd.

Washington: Seattle Children's Hospital/University of Washington, Seattle -- R.L. Gibson, S. McNamara and K. Worrell; University of Washington School of Medicine, Seattle -- S.M. Moskowitz; Deaconess Medical Center, Spokane -- M. McCarthy, C. Llewellyn and S. Wicks.

West Virginia: West Virginia University, Morgantown -- K.S. Moffett and L.S. Baer.

Wisconsin: University of Wisconsin, Adult CF Center, Madison -- G.A. do Pico and L.M. Makholm; University of Wisconsin, CF-Pediatric Pulmonary Center, Madison -- M.J. Rock and S.R. Osmond; Medical College of Wisconsin, Milwaukee -- J. Biller; Children's Hospital of Wisconsin, Milwaukee -- T. Miller.

<u>Argentina</u>

Cystic Fibrosis Center, La Plata -- A. Fernandez and F. Renteria.

<u>Australia</u>

Prince Charles Hospital, Chermside, QLD -- S.C. Bell; Herston Royal Children's Hospital, Brisbane, QLD -- C. Wainwright and P. Lewindon; The Children's Hospital - Westmead, Sydney, NSW -- H. Selvadurai and K. Gaskin.

<u>Belgium</u>

Universitair Ziekenhuis Gent, Gent -- S. Van Biervliet.

<u>Canada</u>

Alberta Children's Hospital, Calgary, Alberta -- M. Montgomery; Adult Cystic Fibrosis Clinic, University of Calgary Medical Clinic of the Foothills Medical Center, Calgary, Alberta -- H.R. Rabin and J. Leong; University of Alberta Hospitals - Paediatric, Edmonton, Alberta -- P. Zuberbuhler; University of Alberta Hospitals - Adult Clinic, Edmonton, Alberta -- N.E. Brown, Adult & Pediatric Clinics -- J. Tabak; BC Children's Hospital, Vancouver, British Columbia -- A.G.F. Davidson; St. Paul's Adult CF Clinic, Vancouver, British Columbia -- E.M. Nakielna; Victoria General Hospital, Victoria, British Columbia -- B. Habbick; Royal Jubilee Hospital - Adult, Victoria, British Columbia -- I. Waters and S. Wiltse; Health Sciences Centre, Winnipeg, Manitoba -- W. Kepron; Children's Hospital of

Winnipeg - Paediatric, Winnipeg, Manitoba -- H. Pasterkamp; Saint John Regional Hospital, Clinic 2, St. John, New Brunswick -- D.N. Garey and G. Bishop; Janeway Child Health and Rehabilitation Centre, St. John's and Health Science Centre, St. John's, Newfoundland -- M. Noseworthy; Queen Elizabeth II Health Sciences Centre, Halifax, Nova Scotia -- R.T. Michael, A.M. Dale and F.A. Gosse; IWK Health Centre, Halifax, Nova Scotia -- W. Robinson; Hospital for Sick Children, Toronto -- P.R. Durie and M. Corey; Hamilton Health Science Corp. - Adults, Hamilton, Ontario -- A. Freitag; Hamilton Health Science Corp. - Paediatric, Hamilton, Ontario -- L. Pedder; Hôtel Dieu Hospital - Paediatric, Kingston, Ontario -- R. Van Wylick; Hotel Dieu Hospital - Adult, Kingston, Ontario -- M.D. Lougheed and L. Kodiattu; Kitchener-Waterloo Health Center of Grand River Hospital - Adult, Kitchener, Ontario -- M. Jackson; Kitchener-Waterloo Health Center of Grand River Hospital - Paediatric, Kitchener, Ontario -- K. Malhotra; London Health Sciences - Children's Hospital of Western Ontario, London, Ontario -- B. Lyttle; London Health Sciences - Adult, London, Ontario -- N.A.M. Paterson; Ottawa General Hospital, Ottawa, Ontario -- S. Aaron; Children's Hospital of Eastern Ontario, Ottawa, Ontario -- M. Boland, T. Kovesi and A. Smith; Sudbury Regional Hospital, Laurentian Site, Sudbury, Ontario -- V.J. Kumar and S. Zinger; St. Michael's Hospital, Toronto, Ontario -- E. Tullis; Complexe Hospitalier de la Sagamie Hôpital de Chicoutimi, Chicoutimi, Quebec -- F. Simard; Sherbrooke - Centre Universitaire de Sante de L'Estrie, Fleurimont, Quebec -- L. Rivard and A. Cantin; Centre Hospitalier Regional de L'Outaouais, Hull, Quebec -- G. Cote; Montreal Children's Hospital of McGill University, Montreal, Quebec -- L.C. Lands; Hôpital Sainte-Justine, Montreal, Quebec -- J.E. Marcotte; Montreal Chest Institute, McGill University Health Centre, Montreal, Quebec -- E. Matouk; Centre Hospitalier de l'Université de Montréal-Hôtel-Dieu, Montreal, Quebec -- Y. Berthiaume, A. Jeanneret and M. Van Spall; Centre Hospitalier de l'Universite Laval, Québec, Quebec -- G. Rivard; Centre Hospitalier Regional de Rimouski, Rimouski, Quebec -- J. Boucher; Centre Hospitalier Rouyn-Noranda, Rouyn-Noranda, Quebec -- N. Petit; Regina General Hospital, Regina, Saskatchewan -- B. Holmes; Royal University Hospital, Saskatoon, Saskatchewan -- D. Cotton and K. Ramlall.

Chile

Facultad de Medicina, Clinica Alemana-Universidad del Desarrollo, Santiago -- G. Repetto.

Czech Republic

CF Center, Charles University, Prague -- M. Macek, Jr., V. Vavrova, J. Bartosova and L. Fila.

France

Centre Hospitalier Universitaire de Bicêtre, Le Kremlin-Bicêtre Cedex -- D. Debray; Hopital Necker-Enfants Malades, Paris -- F. Lacaille; CF Center, University Hospital Robert Debré, Paris -- A. Munck.

Germany

Medizinische Hochschule Hannover, Hannover -- B. Tümmler.

Ireland

Our Lady's Children's Hospital Crumlin, Dublin -- M. Rowland and B. Bourke; Our Lady's Children's Hospital, Crumlin, Dublin -- G. Canny; St. Vincent's University Hospital, Elm Park, Dublin -- C. Gallagher.

Israel

Carmel Hospital, Haifa -- J. Rivlin; Shaare Zedek Medical Center, Jerusalem -- E. Picard; Schneider Children's Hospital, Petach Tikvah -- H. Blau; Hadassah University Organization, Jerusalem -- M. Wilschanski, C. Springer and E. Kerem; Safra Children's Hospital, Tel Hashomer -- Y. Yahav and Y. Bujanover.

Italy

Cystic Fibrosis Center, Pediatric Clinics University of Genova, G. Gaslini Children Hospital, Genoa -- R. Casciaro; Pediatrics, University of Milan, Milan -- C. Colombo; University of Naples Federico II, Naples -- G. Castaldo, F. Salvatore and V. Raia; Centro Fibrosi Cistica, Verona -- M. Cipolli and C. Castellani.

Netherlands

Erasmus University Rotterdam, Rotterdam -- M. Sinaasappel and D. Dooijes.

Scotland

Royal Hospital for Sick Children, Glasgow -- S.C. Ling.

Slovakia

Institute of Molecular Physiology & Genetics, Bratislava -- H. Kayserova.

Turkey

Pediatric Pulmonology, Hacettepe University, Ankara -- U. Ozcelik, N. Kiper and D. Dogru.

United Kingdom

University of Sheffield, Sheffield -- C.J. Taylor and J. McGaw.

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Table 1

Initial study: Characteristics of cystic fibrosis patients with (CFLD) and without (CF no LD) severe liver disease

Variable	Initial study	
	CFLD n = 124	CF no LD n = 843
Age (yrs) [a]		
Mean (± SD)	19.8 (± 7.3)	26.7 (± 9.6)
Median	18.8	23.3
Gender (male)	88 (71.0%)	462 (54.8%)
Caucasian	115 (92.7%)	822 (97.5%)
Genotype [b]		
PI/PI [c]	100 (80.7%)	836 (99.2%)
PI/PS [c]	5 (4.0%)	0 (0.0%)
PS/PS	0 (0.0%)	0 (0.0%)
PI/unknown	16 (12.9%)	7 (0.8%)
unknown/unknown	3 (2.4%)	0 (0.0%)
Meconium ileus [d]	22 (18.2%)	68 (16.5%)
Age of diagnosis of portal hypertension (yrs) [e]		
Mean (± SD)	10.3 (± 5.9)	N/A
Median	10	N/A
Range	0.5 – 26	N/A
Portal hypertension documented by [f]		
Splenomegaly	120 (97.1%)	N/A
Varices (esophageal, rectal)	93 (74.6%)	N/A
Hypersplenism [g]	69 (62.7%)	N/A

[a] Age at time of enrollment.

[b] *CFTR* mutations for CFLD patients in initial study: DF508/DF508 56.5%; DF508/PI 19.4%; DF508/unknown 10.5%; PI/PI 4.8%; PI/unknown 2.4%; PI/PS 4.0%; unknown/unknown 2.4%. *CFTR* mutations for CF no LD patients in initial study: DF508/DF508 92.6%; DF508/PI 5.9%; DF508/unknown 0.7%; PI/PI 0.7%; PI/unknown 0.1%. See eTable 1 for specific *CFTR* genotypes for CFLD patients.

[c] PI = Pancreatic exocrine insufficient mutation, PS = Pancreatic exocrine sufficient mutation.

[d] Data available from 121 CFLD patients (ages 0–26 years) and 411 CF no LD patients (ages 15–28 years).

[e] Data available from 122 CFLD patients.

[f] Documented using several different imaging techniques; some patients had portal hypertension confirmed by more than one method; all patients tested had findings compatible with multi-lobular cirrhosis.

[g] As defined by platelet count < 100,000/ul; data available on 110 patients.

Table 2

Summary of clinical lab values for CF patients with severe liver disease

	Study	Number of Patients [a]	% of patients		
			Normal range	**> 1X to ≤ 2X**	**> 2X**
Aspartate transaminase (AST) (range of values)	Initial	122	23.0	43.4	33.6
	Replication	132	16.7	47.7	35.6
			(≤ 30 U/L)	(31–60 U/L)	(> 60 U/L)
Alanine transaminase (ALT) (range of values)	Initial	116	47.4	35.3	17.2
	Replication	133	44.4	37.6	18.0
			(≤ 40 U/L)	(41–80 U/L)	(> 80 U/L)
Gamma glutamyl transferase (GGT) (range of values)	Initial	110	24.5	16.4	59.1
	Replication	114	19.3	28.1	52.6
			(≤ 30 U/L)	(31–60 U/L)	(> 60 U/L)
Total bilirubin (T Bili) [b] (range of values)	Initial	106	66.0	18.9	15.1
	Replication	111	70.3	17.1	12.6
			(≤ 1.2 mg/dl)	(1.3–2.4 mg/dl)	(> 2.4 mg/dl)
			Normal range	**Low**	**Very Low**
Albumin [c] (range of values)	Initial	104	49.0	42.3	8.7
	Replication	120	56.7	39.2	4.1
			(≥ 3.5 g/dl)	(2.5–3.4 g/dl)	(< 2.5 g/dl)
			Normal range	**Moderately High**	**High**
International normalized ratio (INR) (range of values)	Initial	88	28.4	51.1	20.5
	Replication	90	32.2	47.8	20.0
			(< 1.2)	(1.2–1.5)	(> 1.5)

[a] Number of patients with data available.

[b] Total bilirubin abnormal in 40.9% of patients (9 out of 22) in Initial Study and 38.1% of patients (8 out of 21) in Replication Study, just prior to liver transplant.

[c] Albumin abnormal in 61.9% of patients (13 out of 21) in Initial Study and 50.0% of patients (10 out of 20) in Replication Study, just prior to liver transplant.

Table 3

Initial study: Prevalence of polymorphic genotypes according to CF patients with (CFLD) and without (no CFLD) severe liver disease

Gene	Variant	SNP rs#	Status of liver disease	Geno type	Patients with genotype		Geno type	Patients with genotype		Geno type	Patients with genotype		Number of patients genotyped	P value [a]	OR (95% CI) [b]
					#	%		#	%		#	%			
SERPINA1 (MIM: 107400)	S Allele (T2313A)	17580	CFLD	AA	90	88.2	AT[c]	12	11.8	TT[d]	0	0.0	102	0.16	1.59 (0.83,3.05)
			no CFLD	AA	619	92.6	AT[c]	49	7.3	TT[d]	1	0.1	669		
	Z allele (G4627A)	28929474	CFLD	GG	110	88.7	AG[e]	14	11.3	AA[f]	0	0.0	124	3.3×10^{-6}[g]	4.72 (2.31,9.61)
			no CFLD	GG	741	97.4	AG[e]	20	2.6	AA[f]	0	0.0	761		
ACE (MIM: 106180)	D/I deletion (T2313A)	N/A	CFLD	DD	43	35.0	DI	54	43.9	II	26	21.1	123	0.45	1.11 (0.85,1.44)
			no CFLD	DD	250	37.3	DI	300	44.7	II	121	18.0	671		
GSTP1 (MIM: 134660)	(A1375G)	1695	CFLD	AA	40	41.7	AG	41	42.7	GG	15	15.6	96	0.32	1.17 (0.86,1.61)
			no CFLD	AA	316	43.7	AG	331	45.8	GG	76	10.5	723		
MBL2 (MIM: 154545)	O	N/A	CFLD	AA	69	59.0	AO	42	35.9	OO	6	5.1	117	0.92	0.98 (0.70,1.38)
			no CFLD	AA	384	57.9	AO	248	37.4	OO	31	4.7	663		
	XA/O	N/A	CFLD	Other	95	82.6	XA/O	14	12.2	O/O	6	5.2	115	0.50	1.14 (0.78,1.65)
			no CFLD	Other	567	85.5	XA/O	65	9.8	O/O	31	4.7	663		
TGFB1 (MIM: 190180)	Promoter (C-509T)	1800469	CFLD	CC	44	39.6	CT	52	46.9	TT	15	13.5	111	0.014	1.45 (1.07,1.95)
			no CFLD	CC	413	49.6	CT	356	42.7	TT	64	7.7	833		
	Codon 10 (C29T)	1800470	CFLD	TT	33	29.5	CT	54	48.2	CC	25	22.3	112	2.8×10^{-3}[h]	1.53 (1.16,2.03)
			no CFLD	TT	343	40.7	CT	390	46.4	CC	109	12.9	842		
	Codon 25 (G74C)	1800471	CFLD	GG	93	83.8	GC	18	16.2	CC	0	0.0	111	0.71	1.10 (0.66,1.85)
			no CFLD	GG	592	85.9	GC	92	13.4	CC	5	0.7	689		

[a] All P values were calculated using Cochran-Armitage Trend test of comparisons of the genotypes.

[b] Odds ratio (OR) for each additional copy of the minor allele with 95% confidence interval (CI).

[c] AT is the heterozygous form

[d] TT is the homozygous form of the S allele.

[e] AG is the heterozygous form

[f] AA is the homozygous form of the Z allele.

[g] Bonferroni-corrected p-value = 3.0×10^{-5}.

[h] Bonferroni-corrected p-value = 0.025.

Table 4

Replication study: Characteristics of cystic fibrosis patients with (CFLD) and without (CF no LD) severe liver disease

Variable	Replication study	
	CFLD n = 136	**CF no LD n = 1088**
Age (yrs)[a]		
Mean (± SD)	18.2 (± 6.2)	27.2 (± 9.2)
Median	16.6	25.0
Gender (male)	82 (60.3%)	566 (52.0%)
Caucasian	125 (91.9%)	1066 (98.0%)
Genotype [b]		
PI/PI [c]	116 (85.4%)	1017 (93.5%)
PI/PS [c]	4 (2.9%)	13 (1.2%)
PS/PS	0 (0.0%)	2 (0.2%)
PI/unknown	14 (10.3%)	44 (4.0%)
unknown/unknown	2 (1.4%)	12 (1.1%)
Meconium ileus [d]	31 (23.8%)	62 (18.3%)
Age of diagnosis of portal hypertension (yrs) [e]		
Mean (± SD)	11.0 (± 4.7)	N/A
Median	11	N/A
Range	0.5 – 28	N/A
Portal hypertension documented by [f]		
Splenomegaly	124 (91.1%)	N/A
Varices (esophageal, rectal)	101 (74.2%)	N/A
Hypersplenism [g]	52 (44.4%)	N/A

[a]Age at time of enrollment.

[b]*CFTR* mutations for CFLD patients in replication study: DF508/DF508 45.6%; DF508/PI 32.4%; DF508/unknown 9.6%; PI/PI 7.4%; PI/unknown 0.7%; PI/PS 2.9%; unknown/unknown 1.4%. *CFTR* mutations for CF no LD patients in replication study: DF508/DF508 62.8%; DF508/PI 27.5%; DF508/PS 0.6%; DF508/unknown 3.7%; PI/PI 3.1%, PI/unknown 0.4%, PI/PS 0.6%; PS/PS 0.2%; unknown/unknown 1.1%. See eTable 1 for specific *CFTR* genotypes for CFLD patients.

[c]PI = Pancreatic exocrine insufficient mutation, PS = Pancreatic exocrine sufficient mutation.

[d]Data available from 130 CFLD patients (ages 0–28 years) and 339 CF no LD patients (ages 15–28 years).

[e]Data available from 120 CFLD patients.

[f]Documented using several different imaging techniques; some patients had portal hypertension confirmed by more than one method; all patients tested had findings compatible with multi-lobular cirrhosis.

[g]As defined by platelet count < 100,000/ul; data available on 117 patients.

Table 5

Replication study: Prevalence of polymorphic genotypes according to CF patients with (CFLD) and without (no CFLD) severe liver disease

Gene	Variant	SNP rs#	Status of liver disease	Geno type	Patients with genotype		Geno type	Patients with genotype		Geno type	Patients with genotype		Number of patients genotyped	P value [a]	OR (95% CI) [b]
					#	%		#	%		#	%			
SERPINA1 (MIM: 107400)	Z allele (G4627A)	28929474	CFLD	GG	127	93.4	AG[c]	9	6.6	AA[d]	0	0.0	136	1.4×10^{-3}[e]	3.42 (1.54,7.59)
			no CFLD	GG	1062	98.0	AG[c]	22	2.0	AA[d]	0	0.0	1084		
TGFB1 (MIM: 190180)	Codon 10 (C29T)	1800470	CFLD	TT	51	38.1	CT	62	46.2	CC	21	15.7	134	0.96	1.01 (0.77,1.31)
			no CFLD	TT	290	38.3	CT	349	46.1	CC	118	15.6	757		

[a]All P values were calculated using Cochran-Armitage Trend test of comparisons of the genotypes.

[b]Odds ratio (OR) for each additional copy of the minor allele with 95% confidence interval (CI).

[c]AG is the heterozygous form

[d]AA is the homozygous form of the Z allele

[e]Bonferroni-corrected p-value = 2.8×10^{-3}.