Prevalence and Risk Factors of Significant Fibrosis in Patients With Nonalcoholic Fatty Liver Without Steatohepatitis

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BACKGROUND & AIMS: **In patients with nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH) is a risk factor for the development of fibrosis. However, fibrosis has been observed in livers of patients without NASH. We aimed to estimate the prevalence of fibrosis in patients without NASH and risk factors for fibrosis.**

METHODS: **We analyzed data from 1738 subjects (44.9% with severe obesity) in a cross-sectional liver biopsy cohort enrolled at referral centers in Italy and Finland. Biopsy specimens were analyzed histologically by a blinded pathologist at each center, and a diagnosis of NASH was made based on steatosis (≥5% of hepatocytes), hepatocellular ballooning, and lobular inflammation. We also collected data on demographic features, metabolic comorbidities, and genetic factors, and performed logistic regression analyses. Findings were validated using data from 118 consecutive patients with NAFLD who underwent sequential liver biopsies at tertiary referral centers in Italy.**

RESULTS: **In the cross-sectional cohort, 132 of 389 patients (33.9%) with significant fibrosis had no NASH and 39 patients (10.0%) had no inflammation. The dissociation between NASH and fibrosis was significantly greater in patients with severe obesity ($P < .005$). Steatosis, ballooning, and lobular inflammation each were associated independently with significant fibrosis ($P < .001$); age, adiposity, fasting hyperglycemia, and the *PNPLA3* I148M variant also were associated with fibrosis. In patients without, but not in those with NASH, significant fibrosis was associated with steatosis grade and the *PNPLA3* I148M variant. In patients without NASH, age, fasting hyperglycemia, ballooning, and inflammation were associated with fibrosis. In the validation cohort, 16 of 47 patients (34.0%) with clinically significant fibrosis did not have NASH at baseline. In patients with fibrosis without baseline NASH, worsening of fibrosis (based on later biopsies) was associated with fasting hyperglycemia and more severe steatosis ($P = .016$).**

Abbreviations used in this paper: BMI, body mass index; SF, clinically significant fibrosis; FPR, fibrosis progression rate; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; T2D, type 2 diabetes.

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CONCLUSIONS: **In an analysis of biopsy specimens collected from patients with NAFLD at a single time point, one third of patients with significant fibrosis did not have NASH. We validated this finding in a separate cohort. In patients without NASH, fasting hyperglycemia, severe steatosis, mild inflammation or ballooning, and the *PNPLA3* I148M variant identified those at risk of significant fibrosis.**

Keywords: History; Progression; Risk Factors; Inflammatory Response.

Nonalcoholic fatty liver disease (NAFLD), defined in the presence of increased hepatic fat content not explained by at-risk alcohol intake, has become the leading cause of liver disease.[1] NAFLD is associated with excessive adiposity, insulin resistance, physical inactivity, and qualitative alterations of the diet and microbiota.[2] The factors that drive disease progression are heterogeneous, encompassing multiple hits leading to inflammation and the development of nonalcoholic steatohepatitis (NASH),[3] with genetic factors playing an important role.[4]

NASH is characterized by fatty liver, associated with both hepatocellular damage and lobular inflammation.[5] NASH has been linked with a faster progression of liver fibrosis,[6] and lobular inflammation may be a marker of more severe disease even in patients without NASH.[7] However, fibrosis frequently progresses even in patients without baseline NASH.[6,8,9] In selected cohorts of patients with aggressive disease, ballooning and lobular inflammation predicted fibrosis progression.[10,11] On the other hand, hepatic fat has also been linked with liver fibrosis progression,[12,13] in line with genetic evidence that hepatic lipid accumulation drives secondary inflammation and fibrogenesis.[14]

Although progressive NAFLD appears to be a heterogeneous disease, with variable involvement of genetic defects[4] and metabolic factors[15] in triggering fibrogenesis independently of inflammation, therapeutic studies presently are focused on the resolution of NASH in patients with severe histologic activity.[16] However, the burden of NAFLD that progresses to clinically significant fibrosis (SF) in the absence of NASH, and the risk factors of clinically SF in these patients are not known. The answers to these questions have implications for modeling of disease burden,[17] for the design of therapeutic trials, and for the applicability of results to clinical practice.

The aim of this study was to examine the prevalence of NAFLD with SF not associated with NASH and/or inflammation in a large multicenter cohort and to identify the risk factors for SF in this subgroup. We validated our findings from our multi-center cohort in a separate prospective cohort.

What You Need to Know

Background

Nonalcoholic steatohepatitis (NASH) is the major risk factor for significant fibrosis in patients with nonalcoholic fatty liver disease (NAFLD). It is not clear how many patients with NAFLD develop significant fibrosis without NASH, or what the risk factors for fibrosis are in this subgroup.

Findings

Significant fibrosis was observed in approximately one third of patients with NAFLD in the absence of NASH. In these individuals, fasting hyperglycemia, severe steatosis, mild inflammation/ballooning, and the *PNPLA3* I148M variant identified those at higher risk of significant fibrosis.

Implications for patient care

The severity of hepatic fat accumulation and the presence of the *PNPLA3* I148M variant can identify patients at risk of significant fibrosis even in the absence of NASH.

Patients and Methods

Study Cohorts

Part of the cross-sectional liver biopsy cohort has been described previously.[18] Briefly, a total of 1738 individuals of European descent were consecutively enrolled from Italian and Finnish referral centers. Inclusion criteria were liver biopsy for suspected NASH (steatosis with increased liver enzyme levels, severe insulin resistance, or other risk factors for NASH) or severe obesity (body mass index [BMI] $>$ 40 kg/m^2), presence of NAFLD, and availability of clinical data and consent. Individuals with increased alcohol intake (men, $>$30 g/d; women, $>$20 g/d), viral and autoimmune hepatitis, or other causes of liver disease were excluded. The study conformed to the Declaration of Helsinki and was approved by the Institutional Review Board of the Fondazione Ca' Granda Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) of Milan. The clinical features of the overall cross-sectional liver biopsy cohort and are presented in Supplementary Table 1.

To validate the results prospectively, we took advantage of a previously described multicenter cohort of 118 patients with NAFLD with serial liver biopsies followed at tertiary referral centers in Italy.[9] The clinical features are described in the Supplementary Methods section.

Histologic Evaluation

Slides were coded and read by 1 expert pathologist at each center who was unaware of patients' identity and

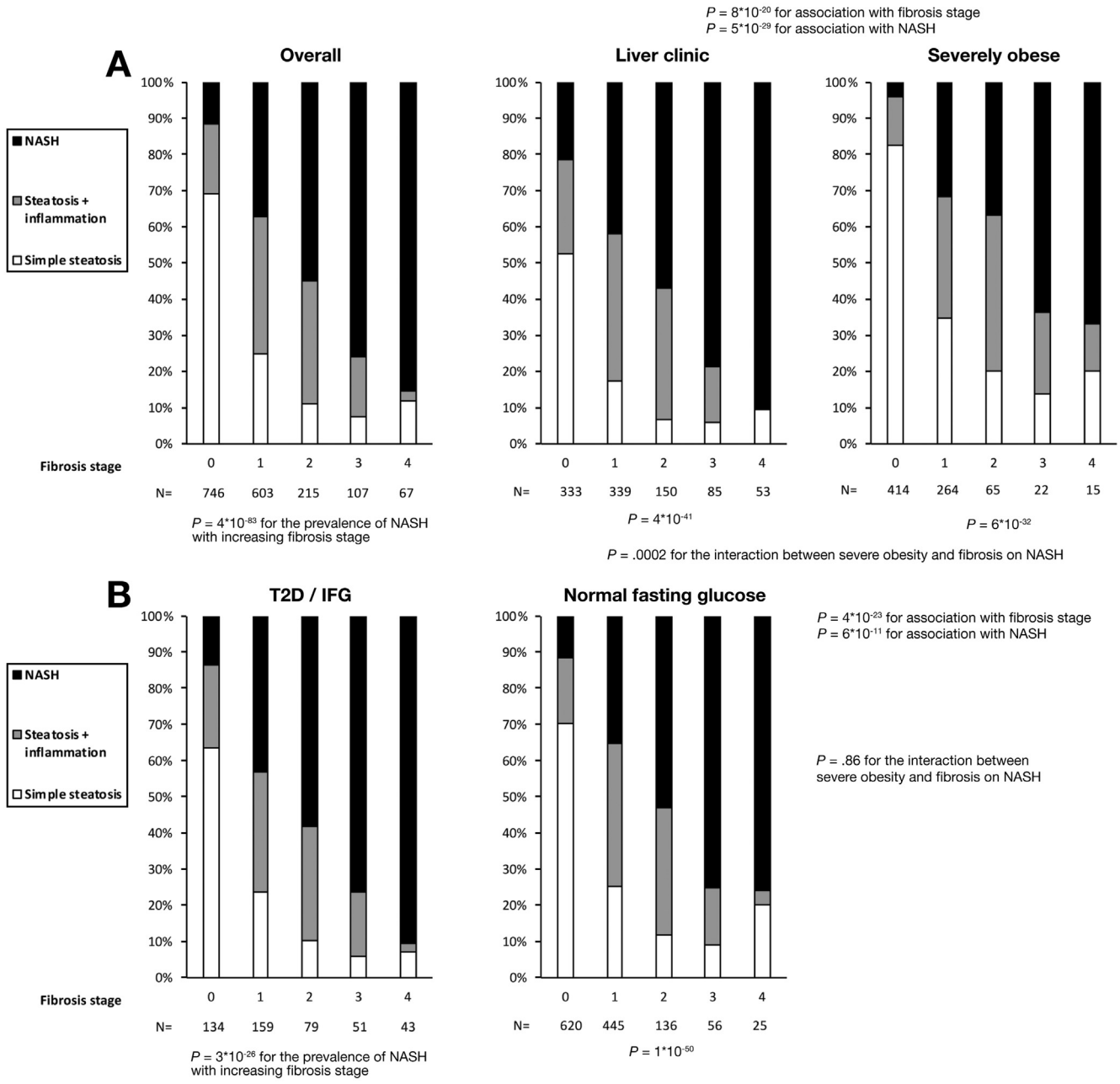

Figure 1. (*A*) Prevalence of nonalcoholic steatohepatitis (NASH) and lobular inflammation not fulfilling NASH criteria, vs simple steatosis according to fibrosis stage in the overall cohort (n = 1738), and in patients stratified by recruitment criterion (referral for liver disease or for severe obesity). (*B*) The same analysis was conducted in patients stratified by the presence of fasting hyperglycemia. T2D, type 2 diabetes; IFG, impaired fasting glucose.

history. Disease activity was assessed according to the NAFLD Activity Score; fibrosis also was staged according to the recommendations of the NAFLD clinical research network.[5] NASH was diagnosed when steatosis (≥5% of hepatocytes), hepatocellular ballooning, and lobular inflammation all were present, irrespective of fibrosis. SF was defined when fibrosis stage was 1 or higher. The concordance between pathologists within this cohort (1 expert pathologist for each center) was very good for fibrosis, and good for steatosis with a coefficient of interobserver agreement for fibrosis, steatosis grade, lobular inflammation, and ballooning of 0.89, 0.76, 0.60, and 0.55, respectively.[19]

Genotyping

When DNA samples and consent were available, the study cohorts were genotyped for rs738409 C>G (*PNPLA3* I148M) by TaqMan 5'-nuclease assays (Life Technologies, Carlsbad, CA), as previously described.[9,18]

Statistical Analysis

Analyses were performed using generalized linear models: linear regression models were fit to analyze

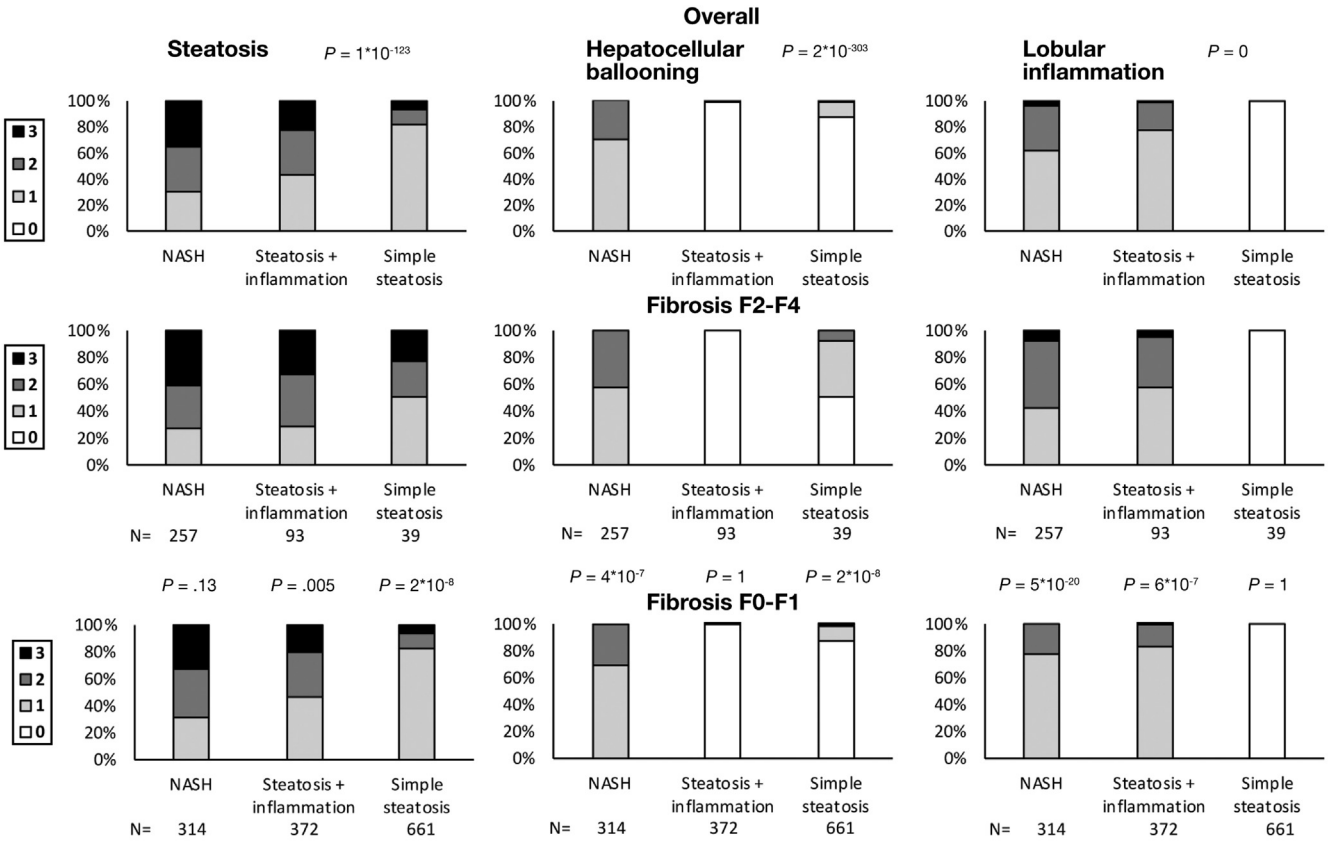

Figure 2. Frequency distribution of the severity of steatosis, ballooning, and lobular inflammation according to the diagnosis of nonalcoholic steatohepatitis (NASH), steatosis plus inflammation, and simple steatosis in the overall cohort and in patients stratified according to the presence of clinically significant fibrosis.

continuous traits (fibrosis progression rate [FPR]), and logistic regression for binary traits (SF). Models were adjusted for confounding factors, as specified. For the evaluation of risk factors of fibrosis, we focused on demographic features (age, sex), specific histologic features of NAFLD (steatosis, ballooning, lobular inflammation), metabolic comorbidities (BMI and impaired fasting glucose/type 2 diabetes [T2D]), which were available in all patients, and genetic factors (*PNPLA3* I148M variant, tested assuming an additive model), available in 1698 patients in the cross-sectional cohort (97.7% of the whole cohort), to assess the factors potentially determining fibrosis development. Participants with missing genetic data were dropped from the final multivariate models. In the prospective cohort, the I148M variant was not considered because it was not available in the majority of patients. Variables with skewed distributions were transformed logarithmically before entering the models. The interaction between NASH and confounding variables in determining the risk of SF was formally tested by entering the product term in the final multivariate models.

Statistical analyses were performed using JMP 12.0 (SAS Institute, Cary, NC) and R statistical analysis software version 3.3.2 (http://www.R-project.org). *P* values less than .05 were considered statistically significant.

Results

Impact of Nonalcoholic Steatohepatitis and Inflammation on Fibrosis in the Cross-Sectional Cohort

Of the overall cohort, 32.9% of patients had NASH and 22.4% had SF. The SF prevalence in patients with and without NASH was 257 of 571 (45.0%) vs 132 of 1165 (11.3%), respectively ($P = 2*10^{-53}$). The histologic activity in patients stratified by fibrosis stage is shown in Supplementary Table 2. The frequency of NASH, steatosis associated with inflammation, and simple steatosis according to fibrosis stage is shown in Figure 1*A* (left panel). Expectedly, there was a trend for an increasing prevalence of NASH with worsening of fibrosis ($P = 4*10^{-83}$). Most patients without fibrosis had simple steatosis. However, as many as 132 of 389 (33.9%) patients with SF had no NASH, and 39 of 389 (10%) patients with SF had no lobular inflammation.

The proportion of patients with SF despite the absence of lobular inflammation at the time of biopsy was higher in severely obese individuals ($P = .0002$) (Figure 1*A*, right panel). NASH was more prevalent in patients with fasting hyperglycemia than in those with normal glucose tolerance, and a lack of inflammation was

Table 1. Clinical Features of the Study Cohorts Stratified by the Presence of NASH, Steatosis Associated With Lobular Inflammation, or Simple Steatosis

	NASH	Steatosis plus inflammation	Simple steatosis	*P* value
Cross-sectional LBC cohort				
N	571 (32.8)	465 (26.8)	702 (40.4)	
Sex, female	230 (40.3)	197 (42.4)	433 (61.7)	<.0001
Age, *y*	44.7 ± 17.0	40.8 ± 16.8	44.7 ± 10.7	<.0001
BMI, *kg/m²*	32.2 ± 7.7	33.5 ± 9.2	37.3 ± 9.0	<.0001
Obesity	292 (51.1)	247 (53.1)	508 (72.4)	<.0001
T2D/IFG, yes	211 (37.0)	119 (25.6)	136 (19.4)	<.0001
Hypertension, yes	166 (34.2)	123 (30.1)	159 (35.4)	.23
Total cholesterol, *mg/dL*	191 ± 45	196 ± 45	187 ± 43	.0036
Triglyceride level, *mg/dL*	147 ± 77	140 ± 79	131 ± 74	.0017
HDL cholesterol, *mg/dL*	47 ± 14	48 ± 14	48 ± 14	.65
ALT level, *IU/L*	59 (38–88)	48 (26–76)	30 (19–45)	<.0001
AST level, *IU/L*	39 (28–54)	30 (21–46)	22 (17–31)	<.0001
SF, yes	257 (45.0)	93 (20.0)	39 (5.6)	<.0001
Severe fibrosis, stages F3–F4	139 (24.3)	20 (4.3)	15 (2.1)	<.0001
PNPLA3, 148M/M[a]	107 (19.0)	74 (16.2)	53 (7.8)	<.0001
Prospective cohort				
N	49 (41.5)	46 (39.0)	23 (19.5)	
Sex, female	21 (42.9)	15 (32.6)	9 (39.1)	.58
Age, *y*	47.9 ± 13.2	46.4 ± 12.0	47.5 ± 12.5	.80
BMI, *kg/m²*	30.0 ± 4.1	31.1 ± 1.0	30.6 ± 1.4	.72
Obesity	24 (49.0)	19 (41.3)	10 (43.5)	.75
T2D/IFG, yes	28 (58.3)	14 (30.4)	4 (17.4)	.001
Hypertension, yes	20 (40.8)	10 (21.7)	8 (34.8)	.13
LDL cholesterol, *mg/dL*	115 ± 36	125 ± 43	133 ± 33	.16
Triglyceride level, *mg/dL*	136 ± 70	142 ± 86	109 ± 47	.21
HDL cholesterol, *mg/dL*	48 ± 15	46 ± 12	50 ± 15	.50
ALT level, *IU/L*	57 (42–95)	51 (26–82)	35 (21–63)	.78
AST level, *IU/L*	37 (27–62)	34 (22–40)	24 (19–39)	.52
SF, yes	31 (63.3)	15 (32.6)	1 (4.4)	<.0001
Severe fibrosis, stages F3–F4	19 (38.8)	8 (17.4)	0	<.0001
FPR, stages/y	-0.011 ± 0.055	+0.005 ± 0.040	+0.002 ± 0.011	.16
Fibrosis progression, yes	15 (30.6)	12 (26.1)	3 (13.0)	.13
PNPLA3, 148M/M[b]	12 (44.4)	11 (28.2)	2 (8.7)	.0061

NOTE. Values are reported as means ± SD, median {interquartile range}, or number (%), as appropriate. Characteristics of participants were compared using a linear regression model or a logistic regression model (as required).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FPR, fibrosis progression rate; HDL, high-density lipoprotein; IFG, impaired fasting glucose; LBC, liver biopsy cohort; LDL, low-density lipoprotein; NASH, nonalcoholic steatohepatitis; SF, clinically significant fibrosis; T2D, type 2 diabetes.

[a]The *PNPLA3* I148M genotype was available in a subset of 1698 patients.

[b]The *PNPLA3* I148M genotype was available in a subset of 89 patients.

observed in 4 of 24 (16.7%) cirrhotic patients with normal glucose tolerance (Figure 1*B*). Despite the analysis being limited by the reduced sample size, there was no association between NASH and fibrosis in developmental age patients (Supplementary Figure 1*A*), whereas the association between NASH and fibrosis was not influenced significantly by sex (Supplementary Figure 1*B*), or by carriage of the *PNPLA3* I148M variant (Supplementary Figure 1*C*).

Histologic Predictors of Significant Fibrosis in the Cross-Sectional Cohort

Histologic features of NAFLD in patients in the cross-sectional cohort stratified by the severity of liver damage are shown in Figure 2 (upper panel). NASH was characterized by the presence of the most severe grade of steatosis, ballooning, and lobular inflammation, whereas steatosis plus inflammation had an intermediate steatosis and inflammatory grade, and simple steatosis had the lowest grade of steatosis and, by definition, no inflammation.

The clinical features of patients stratified by the presence of NASH or of lobular inflammation are presented in Table 1 (upper panel). There was a progressive increase in the prevalence of impaired fasting glucose/T2D and higher triglyceride levels with increasing disease activity (from simple steatosis, to steatosis plus inflammation, and next NASH), as well as a progressive increase in the frequency of homozygosity of the *PNPLA3* I148M variant.

Table 2. Independent Predictors of Clinically Significant Fibrosis (Stages F2–F4) in the Overall Cross-Sectional LBC Cohort and in Patients Stratified According to Modality of Enrollment (Liver Clinic Vs Severe Obesity)

	Overall			Liver clinic			Severe obesity		
	Estimate	SE	*P* value	Estimate	SE	*P* value	Estimate	SE	*P* value
Sex, female	−0.143	0.078	.061	+0.014	0.009	.88	-0.400	0.106	.0018
Age, *y*	+0.037	0.005	$7*10^{-14}$	+0.040	0.005	$7*10^{-13}$	+0.014	0.011	.23
BMI, kg/m^2	+0.038	0.015	.009	+0.054	0.023	.021	+0.022	0.020	.24
T2D/IFG, yes	+0.480	0.079	$1*10^{-9}$	+0.503	0.098	$8*10^{-8}$	+0.350	0.129	.0067
Steatosis, grade	+0.399	0.079	$3*10^{-7}$	+0.338	0.098	$6*10^{-4}$	+0.547	0.139	$9*10^{-5}$
Ballooning, grade	+0.608	0.103	$4*10^{-9}$	+0.571	0.113	$5*10^{-7}$	+0.776	0.266	.0036
Lobular inflammation, grade	+0.779	0.081	$2*10^{-21}$	+0.733	0.098	$2*10^{-13}$	+0.772	0.266	$2*10^{-7}$
PNPLA3, I148M alleles[a]	+0.326	0.101	.0001	+0.231	0.125	.064	+0.550	0.183	.0036

NOTE. Characteristics of participants were compared using linear regression models. Results in the overall cohort were adjusted for enrollment criterion (liver clinic vs severe obesity), and the covariates shown.
BMI, body mass index; IFG, impaired fasting glucose; LBC, liver biopsy cohort; T2D, type 2 diabetes.
[a]The *PNPLA3* I148M genotype was available in a subset of patients (n = 1698).

The independent predictors of SF are presented in Table 2. SF was associated independently with each feature of NAFLD activity (steatosis, hepatocellular ballooning, and lobular inflammation) after adjustment for confounders, both in the overall cohort and in the subcohorts. Besides NASH features, fasting hyperglycemia and the *PNPLA3* I148M variant increased the risk, the independent impact of the latter being larger in severely obese patients. Furthermore, SF was associated with older age and adiposity in the overall cohort and in the liver clinic subgroup, but not in severely obese individuals. Conversely, female sex was protective in severely obese individuals, although there was no difference in the mean age in females according to recruitment criterion (44.5 ± 19 y in severely obese vs 45.2 ± 9.9 y in liver clinic patients; $P = .58$).

Predictors of Significant Fibrosis According to Nonalcoholic Steatohepatitis in the Cross-Sectional Cohort

The frequency distribution of the histologic grade of steatosis, hepatocellular ballooning, and lobular inflammation between patients who had developed SF or not, stratified by the presence of NASH, steatosis plus inflammation, or simple steatosis, is shown in Figure 2 (bottom panel). In patients with NASH, steatosis grade tended to be severe independently of fibrosis. On the other hand, steatosis was more severe in patients with steatosis and inflammation and in those with steatosis alone who developed SF. SF was associated with ballooning in patients with NASH and simple steatosis, and with more severe inflammation in those with NASH and steatosis plus inflammation.

The independent predictors of SF in patients stratified by the presence of NASH are presented in Table 3. In patients with NASH, SF was associated with more severe ballooning and inflammation, and with older age and fasting hyperglycemia. In patients without NASH, SF was associated with more severe steatosis, inflammation, and fibrosis, and with older age, fasting hyperglycemia, and the *PNPLA3* I148M variant. Steatosis grade and the *PNPLA3* I148M variant seemed to have a relatively greater effect on SF in patients not fulfilling NASH criteria. Even at univariate analysis, the *PNPLA3* I148M variant had a larger effect size on SF in patients without (estimate, +0.552, 0.132; $P = 3*10^{-5}$), than in those with NASH (estimate, +0.239, 0.118; $P = .003$). Results were similar when patients were stratified further by the presence of severe obesity (Supplementary Table 3). In particular, steatosis grade and the *PNPLA3* I148M variant were associated independently with SF in patients without, but not in those with NASH in both subcohorts.

When patients without NASH were stratified by the presence of lobular inflammation, in those with inflammation, SF was associated with steatosis and inflammatory grade, and with older age, male sex, fasting hyperglycemia, and the *PNPLA3* I148M variant. In patients without inflammation, SF was associated with more severe steatosis and ballooning, and with adiposity and the *PNPLA3* I148M variant.

Additional sensitivity analyses are provided in the Supplementary Results section (see also Supplementary Tables 4–6); results generally were consistent with those observed in the whole cohort.

Predictors of Fibrosis Evolution According to Nonalcoholic Steatohepatitis in the Prospective Cohort

The baseline clinical features of patients included in the prospective cohort stratified by liver disease activity are reshown in Table 1 (bottom panel). The independent

Table 3. Independent Predictors of Clinically Significant Fibrosis (Stages F2–F4) in the Cross-Sectional LBC Patients Stratified According to Disease Activity

	NASH						No NASH					
	Overall				Steatosis plus inflammation			Simple steatosis				
	OR (95% CI)	Estimate	SE	*P* value	OR (95% CI)	Estimate	SE	*P* value	OR (95% CI)	Estimate	SE	*P* value
Sex, female	079 (0.52–1.20)	-0.074	0.110	.51	0.56 (0.36–0.88)	-0.279	0.116	.016	0.58 (0.38–0.98)	-0.306	0.145	.035	0.56 (0.36–0.88)	-0.171	0.196	.38
Age, *y*	1.05 (1.03–1.06)	+0.042	0.007	$2*10^{-9}$	1.02 (1.01–1.04)	+0.024	0.007	.001	1.02 (1.01–1.04)	+0.023	0.008	.004	1.03 (1.01–1.07)	+0.033	0.017	.051
BMI, kg/m^2	1.04 (0.99–1.08)	+0.029	0.023	.21	1.01 (0.99–1.03)	+0.035	0.020	.087	1.01 (0.97–1.05)	+0.014	0.027	.61	1.03 (1.00–1.05)	+0.070	0.030	.021
T2D/IFG, yes	2.12 (1.39–3.22)	+0.449	0.113	$9*10^{-5}$	2.13 (1.36–3.32)	+0.387	0.113	$6*10^{-4}$	1.95 (0.91–4.23)	+0.413	0.143	.004	2.31 (1.32–4.06)	+0.318	0.196	.10
Steatosis, grade	1.18 (0.74–1.87)	+0.033	0.122	.79	3.02 (1.96–4.65)	+0.585	0.111	$9*10^{-8}$	2.50 (1.20–5.17)	+0.337	0.186	.010	2.09 (1.18–3.71)	+0.694	0.183	$2*10^{-4}$
Ballooning, grade	2.14 (1.37–3.34)	+0.365	0.115	.001	2.56 (1.41–4.65)	+1.342	0.432	.002	-	-	-	-	5.84 (273–12.5)	+1.556	0.459	$7*10^{-4}$
Lobular inflammation, grade	4.24 (2.81–6.40)	+0.710	0.109	$8*10^{-11}$	5.03 (2.96–8.53)	+0.718	0.134	$9*10^{-8}$	5.03 (2.96–8.53)	+0.596	0.140	$2*10^{-5}$	-	-	-	-
PNPLA3, I148M alleles[a]	1.21 (0.93–1.60)	+0.211	0.143	.14	1.61 (1.20–2.15)	+0.442	0.149	.003	1.61 (1.20–2.15)	+0.359	0.183	.038	1.45 (1.02–2.08)	+0.568	0.248	.022

NOTE. Characteristics of participants were compared using linear regression models. All results were adjusted for enrollment criterion (liver clinic vs severe obesity) and the covariates are shown. For ordinal variables (histologic features of liver damage), variables were dichotomized and ORs were reported for the presence of steatosis grade higher than 1, ballooning >1, and lobular inflammation greater than 1.
BMI, body mass index; IFG, impaired fasting glucose; LBC, liver biopsy cohort; NASH, nonalcoholic steatohepatitis; OR, odds ratio; T2D, type 2 diabetes.
[a]The *PNPLA3* I148M genotype was available in a subset of patients (n = 1698).

predictors of FPR in the overall prospective cohort and in patients stratified according to disease activity are shown in Table 4. In the overall cohort, faster FPR was associated with fasting hyperglycemia, but not with other severe features of liver damage. After stratification for the presence of NASH, in patients with NASH, faster FPR was associated with male sex and more severe ballooning. In patients without NASH, faster FPR was associated independently with fasting hyperglycemia and steatosis grade.

In patients without baseline NASH who had fibrosis progression at follow-up evaluation, we observed a higher rate of NASH diagnosis at follow-up evaluation as compared with nonprogressors (6 of 15 [40.0%] vs 8 of 54 [14.8%]; $P = .043$), whereas the variation in BMI, liver enzyme levels, and T2D incidence were not significantly different (not shown).

Discussion

In a large European multicenter cohort of patients with NAFLD, we examined the prevalence and risk factors of SF in the absence of histologic NASH. We found that the prevalence of SF in the absence of NASH was much higher than that estimated by recent epidemiologic studies. Furthermore, risk factors for SF were different in patients with compared with patients without NASH, in which a larger role was played by steatosis severity and the *PNPLA3* I148M variant.

More than one third of SF patients did not have NASH, and more than 1 in 10 did not have any sign of histologic inflammation. Although the association between NASH and SF was influenced by severe obesity, in that individuals referred for this condition had a higher prevalence of SF in the absence of NASH, 27% of patients referred to a liver clinic with SF did not have NASH, and 7% had no inflammation. Approximately 10% of patients with cirrhosis had no signs of lobular inflammation, possibly owing to burnt-out disease in a fraction of these individuals.[20] However, the prevalence of SF in the absence of NASH was higher in patients with fibrosis stages 2 and 3 than in those with cirrhosis, and in patients without NASH, SF was associated with more severe steatosis, which would not be in line with burnt-out disease. It is possible that these individuals were experiencing a phase of temporary remission of disease activity at the time of biopsy, leading to reduced ballooning and inflammation, but without improvement in fibrosis. Notwithstanding, the prevalence of SF in the absence of NASH in this study was several-fold higher than that estimated in recent epidemiologic projections of the burden of NAFLD (approximately 5%),[17] suggesting that the disease evolution may have been underestimated.[17]

The higher prevalence of SF in the absence of NASH in severely obese patients may be explained by the direct impact of insulin resistance in disease progression

Table 4. Independent Predictors of Fibrosis Progression Rate in the Overall NAFLD Prospective Cohort (n = 118) and Stratified According to the Presence of NASH

	Overall			NASH			No NASH		
	Estimate	SE	*P* value	Estimate	SE	*P* value	Estimate	SE	*P* value
Sex, female	+0.001	0.004	.74	−0.014	0.007	.032	+0.001	0.004	.30
Age, *y*	+0.001	0.001	.35	+0.001	0.001	.31	+0.001	0.001	.93
T2D/IFG, yes	+0.013	0.005	.014	+0.009	0.009	.38	+0.013	0.005	$3.6*10^{-5}$
Steatosis, grade >1	+0.014	0.010	.22	+0.005	0.015	.89	+0.021	0.010	.016
Ballooning, grade >0	-0.019	0.009	.047	+0.036	0.014	.012	+0.015	0.017	.36
Lobular inflammation, grade >1	+0.010	0.010	.55	+0.024	0.016	.14	+0.001	0.001	.66

NOTE. Characteristics of participants were compared using linear regression models. All results were adjusted for the covariates shown in the table, and for baseline fibrosis stage.
IFG, impaired fasting glucose; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; T2D, type 2 diabetes.

independent of inflammation.[15,21] Alternatively, the more prolonged fasting advised before undergoing bariatric surgery than before liver biopsy performed in outpatients may have improved disease activity in severely obese patients, partially masking the contribution of inflammation to SF development in this subgroup. Indeed, little is known about the impact of the duration of fasting as well as of the quality of diet in the period immediately preceding evaluation of liver damage (either by liver biopsy or by noninvasive approaches) on disease activity. Such information would allow a better interpretation of the results and standardization of the procedures.

Second, we found that each of the individual major features of NASH, namely steatosis, ballooning, and lobular inflammation, was independently associated with SF. These data suggest that they reflect different pathophysiological aspects of the disease (eg, fat accumulation, oxidative stress, and inflammation), which contribute to disease pathogenesis.[14] Furthermore, the impact of fasting hyperglycemia and severe insulin resistance was independent of the histologic activity of the disease,[15,22] suggesting that it may be mediated directly by glucose toxicity or alteration in insulin signaling pathways.[15,21,23] In addition, it is worth noting that the *PNPLA3* I148M variant was associated with SF independently of the well-established impact on histologic fat and inflammation, in keeping with a further direct effect of this mutation on fibrogenesis by acting on retinol/lipid metabolism in hepatic stellate cells.[24–27]

Importantly, the risk factors of SF were different according to the presence of NASH. Indeed, hepatocellular ballooning and inflammation, as well as fasting hyperglycemia and aging, were associated with SF irrespective of the presence of NASH. On the other hand, an independent impact of steatosis grade, as well as of male sex, and of the *PNPLA3* I148M variant was specifically observed in patients without NASH. In keeping, prospective cohort studies showed that in patients with severe NASH disease progression was predicted by ballooning or inflammation,[10,11] while in unselected cohorts of patients with and without NASH disease progression was predicted by steatosis grade.[12,13] This observation may be explained by the fact that patients with NASH already have severe fat accumulation and activation of fibrogenic pathways, masking the role of hepatic fat accumulation in triggering liver disease.

The association between steatosis grade and fibrosis in patients without NASH also was replicated in a prospective cohort. Here, we confirmed that during follow-up evaluation fibrosis progression occurred in as much as 50% of cases without NASH at baseline, and in 10% of cases without baseline histologic inflammation. However, in 40% of cases without baseline NASH who had fibrosis progression, this was associated with the development of NASH during follow-up evaluation, suggesting that they may be affected by a remitting–relapsing form of disease, which might have led to underestimation of the disease activity at the time of baseline histologic evaluation. Therefore, fluctuation of disease activity during the natural history of NAFLD owing to transient modifications of environmental triggers may contribute to the frequent presence of clinically significant fibrosis in the absence of NASH. Heterogeneity of liver damage distribution and sampling variability (affecting both disease activity grading and fibrosis staging), and/or interobserver variability in histologic scoring, especially concerning the diagnosis of ballooning, should be considered as possible explanations.

Despite including a large series of patients, with validation in a prospective cohort, this study had limitations. These include the retrospective design, and the consequent impossibility to have a centralised reevaluation of all slides. However, histologic evaluations were performed by expert pathologists at tertiary referral centers, receiving regular feedback from central histologic readings in randomized controlled trials, and the concordance for staging fibrosis and steatosis was good.[19] We acknowledge that the concordance for evaluation of inflammation and ballooning, which are key for

the diagnosis of NASH, was only moderate.[19] Notwithstanding, in the present study these histologic features turned out to represent the most accurate SF predictors in all subgroups, rendering it unlikely that inaccurate staging may account for the present findings. Furthermore, this limitation was partially compensated by the large sample size, suggesting that results reflect the clinical challenges in identifying at-risk patients in referral center. Finally, genetic data were not available in a subgroup, and results may not be applicable to non-European individuals.

In conclusion, in a large multicenter cohort of patients with NAFLD, we found that a third of patients with SF did not have histological evidence of NASH. One out of 10 patients with SF did not have any signs of inflammatory activity. In addition, the dissociation between NASH and fibrosis is more marked in severely obese individuals. Importantly, the severity of steatosis and the *PNPLA3* I148M variant predicted SF, and steatosis predicted fibrosis progression specifically in patients without NASH. These findings suggest that a higher than expected proportion of patients with NAFLD may evolve toward SF, possibly owing to fluctuating disease activity, which is not easily captured at a definite time point, and/or the existence of mechanisms of disease progression that may be partially different in different subgroups.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2019.01.027.

References

1. Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 2016;64:73–84.
2. Valenti L, Bugianesi E, Pajvani U, et al. Nonalcoholic fatty liver disease: cause or consequence of type 2 diabetes? Liver Int 2016;36:1563–1579.
3. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. Hepatology 2010;52:1836–1846.
4. Eslam M, Valenti L, Romeo S. Genetics and epigenetics of NAFLD and NASH: clinical impact. J Hepatol 2018;68:268–279.
5. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–1321.
6. Singh S, Allen AM, Wang Z, et al. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. Clin Gastroenterol Hepatol 2015;13:643–654.e1–9; quiz e39–40.
7. Pais R, Charlotte F, Fedchuk L, et al. A systematic review of follow-up biopsies reveals disease progression in patients with non-alcoholic fatty liver. J Hepatol 2013;59:550–556.
8. McPherson S, Hardy T, Henderson E, et al. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: implications for prognosis and clinical management. J Hepatol 2015;62:1148–1155.
9. Pelusi S, Petta S, Rosso C, et al. Renin-angiotensin system inhibitors, type 2 diabetes and fibrosis progression: an observational study in patients with nonalcoholic fatty liver disease. PLoS One 2016;11:e0163069.
10. Sanyal A, Harrison S, Ratziu V, et al. Changes in fibrosis, but not the NAFLD Activity Score (NAS), are associated with disease progression in patients with nonalcoholic steatohepatitis (NASH) and advanced fibrosis. J Hepatol 2017;66:S2.
11. Ratziu V, Wong VW-S, Lanthier N, et al. Hepatic fibrosis is associated with histological activity in nonalcoholic steatohepatitis: an analysis from a large database of screening biopsies in the CENTAUR trial. J Hepatol 2017;66:S113.
12. Ajmera V, Park CC, Caussy C, et al. Magnetic resonance imaging proton density fat fraction associates with progression of fibrosis in patients with nonalcoholic fatty liver disease. Gastroenterology 2018;155:307–310 e2.
13. McPherson S, Pais R, Valenti L, et al. Further delineation of fibrosis progression in NAFLD: evidence from a large cohort of patients with sequential biopsies. J Hepatol 2017;64:S593.
14. Dongiovanni P, Stender S, Pietrelli A, et al. Causal relationship of hepatic fat with liver damage and insulin resistance in nonalcoholic fatty liver. J Intern Med 2018;283:356–370.
15. Dongiovanni P, Meroni M, Baselli GA, et al. Insulin resistance promotes lysyl oxidase like 2 induction and fibrosis accumulation in non-alcoholic fatty liver disease. Clin Sci 2017;131:1301–1315.
16. Sanyal AJ, Brunt EM, Kleiner DE, et al. Endpoints and clinical trial design for nonalcoholic steatohepatitis. Hepatology 2011;54:344–353.
17. Estes C, Anstee QM, Teresa Arias-Loste M, et al. Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016-2030. J Hepatol 2018;69:896–904.
18. Mancina RM, Dongiovanni P, Petta S, et al. The MBOAT7-TMC4 variant rs641738 Increases risk of nonalcoholic fatty liver disease in individuals of European descent. Gastroenterology 2016;150:1219–1230 e6.
19. Petta S, Valenti L, Marra F, et al. MERTK rs4374383 polymorphism affects the severity of fibrosis in non-alcoholic fatty liver disease. J Hepatol 2016;64:682–690.
20. van der Poorten D, Samer CF, Ramezani-Moghadam M, et al. Hepatic fat loss in advanced nonalcoholic steatohepatitis: are alterations in serum adiponectin the cause? Hepatology 2013;57:2180–2188.
21. Valenti L, Mendoza RM, Rametta R, et al. Hepatic Notch signaling correlates with insulin resistance and nonalcoholic fatty liver disease. Diabetes 2013;62:4052–4062.
22. Marchesini G, Bugianesi E, Forlani G, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology 2003;37:917–923.
23. Paradis V, Perlemuter G, Bonvoust F, et al. High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: a potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. Hepatology 2001;34:738–744.
24. Pirazzi C, Valenti L, Motta BM, et al. PNPLA3 has retinyl-palmitate lipase activity in human hepatic stellate cells. Hum Mol Genet 2014;23:4077–4085.

25. Mondul A, Mancina RM, Merlo A, et al. PNPLA3 I148M variant influences circulating retinol in adults with nonalcoholic fatty liver disease or obesity. J Nutr 2015;145:1687–1691.
26. Pingitore P, Dongiovanni P, Motta BM, et al. PNPLA3 overexpression results in reduction of proteins predisposing to fibrosis. Hum Mol Genet 2016;25:5212–5222.
27. Bruschi FV, Claudel T, Tardelli M, et al. The PNPLA3 I148M variant modulates the fibrogenic phenotype of human hepatic stellate cells. Hepatology 2017;65:1875–1890.

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Conflicts of interest
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Supplementary Methods

Part of the cross-sectional liver biopsy cohort has been described previously.[1,2] Briefly, a total of 1738 individuals of European descent were consecutively enrolled from Italian and Finnish referral centers. Inclusion criteria were a liver biopsy for suspected NASH or severe obesity, the presence of NAFLD, and availability of clinical data and consent. Individuals with increased alcohol intake (men, >30 g/d; women, >20 g/d), viral and autoimmune hepatitis, or other causes of liver disease were excluded. The study conformed to the Declaration of Helsinki and was approved by the Institutional Review Board of the Fondazione Ca' Granda Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) of Milan. The clinical features of the overall cross-sectional liver biopsy cohort and after stratification for the enrollment criterion is presented in Supplementary Table 1.

To validate the results prospectively, we took advantage of a previously described multicenter cohort of 118 patients with NAFLD with serial liver biopsies followed up at tertiary referral centers in Italy.[3] Their clinical features have been described previously in detail.[3] Briefly, they were mostly middle-aged men or postmenopausal women, overweight or obese, with a high prevalence of metabolic alterations defining metabolic syndrome and/or altered liver enzyme levels. The median follow-up period was 36 months (interquartile range, 24–77 mo). The FPR was calculated by taking the ratio between the difference of fibrosis stage and the time (in months) between the baseline and follow-up biopsy, and it was treated as a continuous variable. Forty-nine patients (41.5%) had NASH, 46 (39.0%) had steatosis plus inflammation, and 23 (19.5%) had simple steatosis.

Histologic Evaluation

Slides were coded and read by 1 expert pathologist at each center who was unaware of patients' identity and history. A minimum 15-mm length of biopsy specimen or the presence of at least 10 complete portal tracts was required.[4] Steatosis was graded based on the percentage of affected hepatocytes as follows: 0, 0% to 5%; 1, 6% to 32%; 2, 34% to 66%; and 3, 67% to 100%. Disease activity was assessed according to the NAFLD Activity Score, with systematic evaluation of hepatocellular ballooning and lobular inflammation; fibrosis also was staged according to the recommendations of the NAFLD clinical research network.[5] NASH was diagnosed when steatosis, hepatocellular ballooning, and lobular inflammation all were present. The concordance between pathologists within this cohort was very good for fibrosis, and good for steatosis with a coefficient of interobserver agreement for fibrosis, steatosis grade, lobular inflammation, and ballooning of 0.89, 0.76, 0.60, and 0.55, respectively.[6]

Supplementary Results

Sensitivity Analyses

The independent predictors of advanced fibrosis (stages F3–F4) in patients stratified by the presence of NASH are shown in Supplementary Table 3. Although the development of advanced fibrosis tends to be associated with a reduction of liver fat and disease activity, and that the power for this analysis was limited by the relatively low number of patients with advanced fibrosis without NASH included in the present cohort, steatosis grade remained nearly associated with advanced fibrosis in patients without NASH, independently of confounders, although the association was not statistically significant ($P = .068$). On the other hand, the *PNPLA3* I148M variant remained associated with advanced fibrosis in both patients with and without NASH independently of the histologic and metabolic features of the disease.

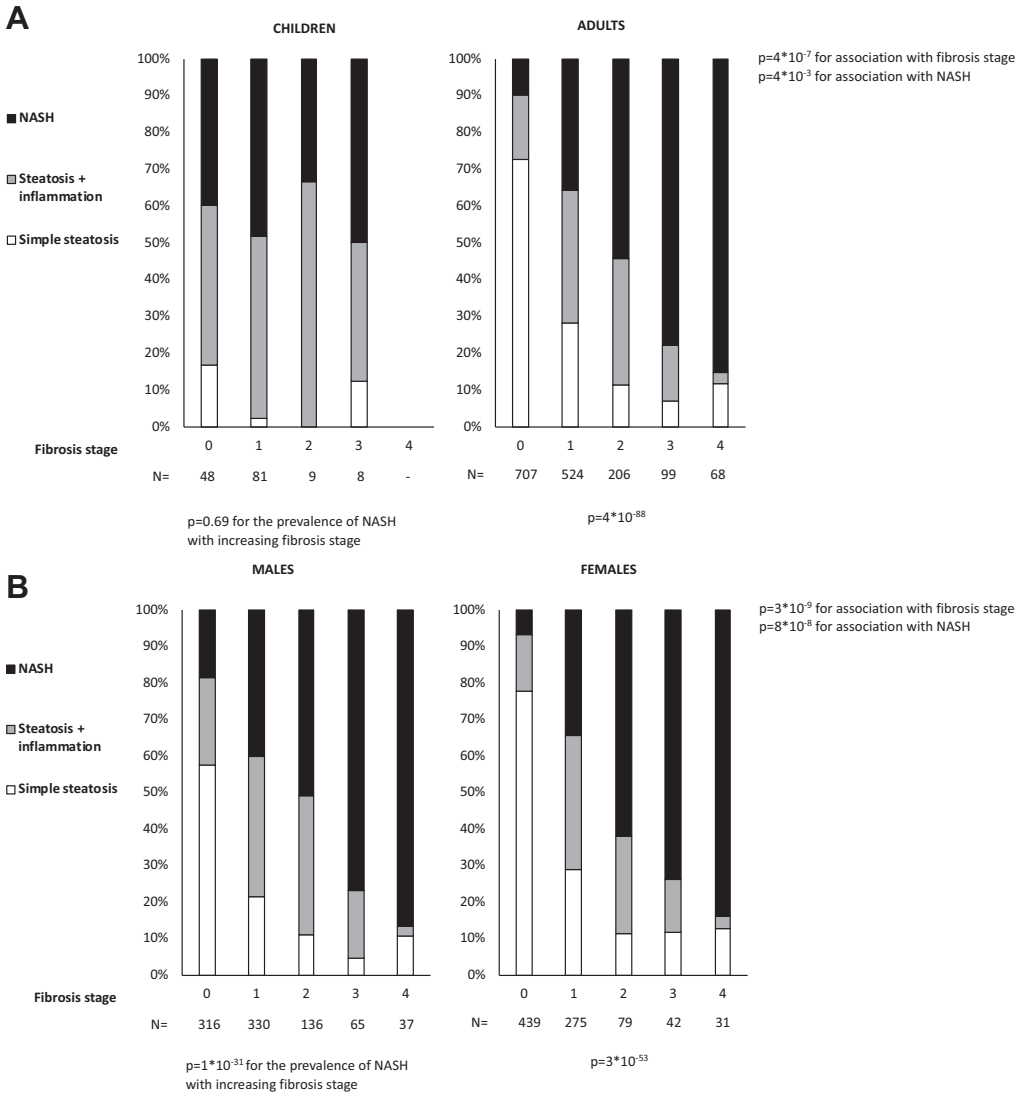
The independent predictors of SF after the exclusion of patients with cirrhosis (stages F2–F3) in those patients stratified by the presence of NASH are shown in Supplementary Table 4. In keeping with what we observed in the whole cohort, steatosis grade and the *PNPLA3* I148M variant were specific independent predictors of clinically significant fibrosis in patients without NASH. These data are consistent with hepatic fat and the *PNPLA3* I148M variant being a driver of the development of liver fibrosis in patients without NASH.

The independent predictors of clinically significant fibrosis in patients without NASH stratified by the presence of T2D are shown in Supplementary Table 5. Steatosis grade was the strongest independent predictor of SF in patients without T2D; SF also was associated with the *PNPLA3* I148M variant, male sex, and lobular inflammation. Despite the relatively low sample size, in patients with T2D, SF remained independently associated with steatosis grade, together with lobular inflammation and male sex. These data confirm that steatosis grade is an independent determinant of SF in patients without NASH irrespective of the presence of T2D.

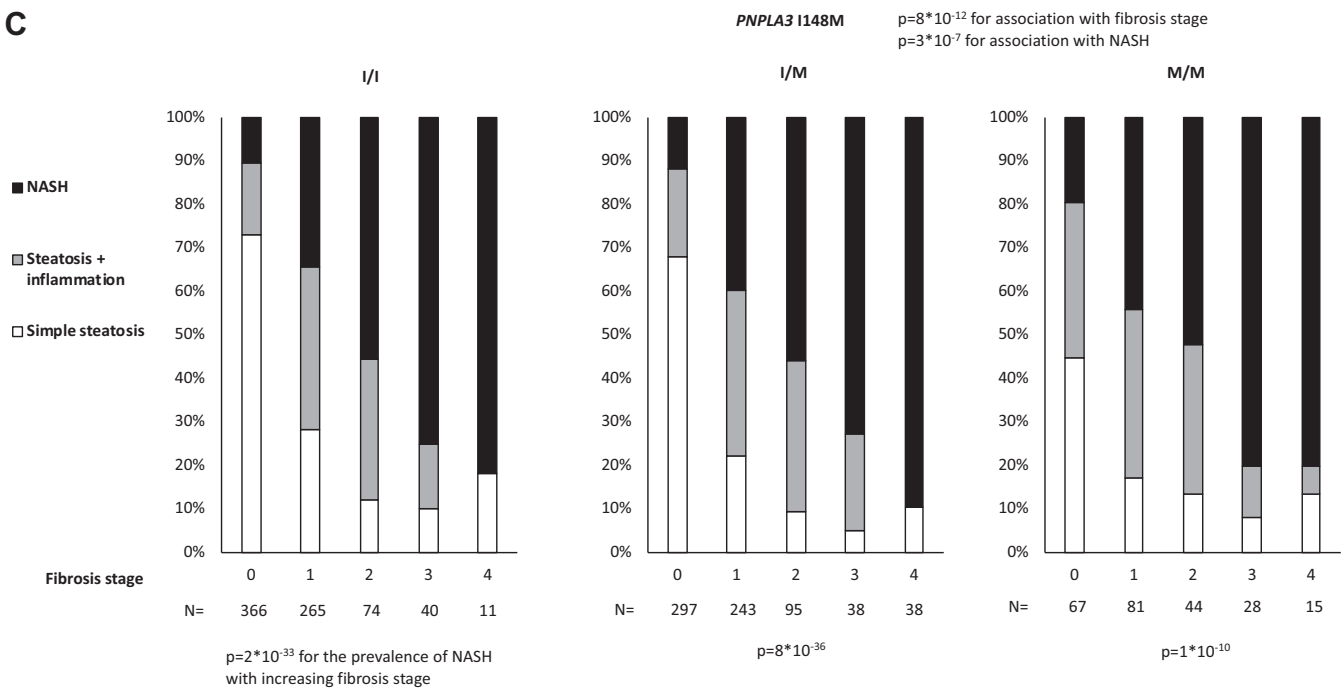
References

1. Dongiovanni P, Petta S, Maglio C, et al. Transmembrane 6 superfamily member 2 gene variant disentangles nonalcoholic steatohepatitis from cardiovascular disease. Hepatology 2015; 61:506–514.
2. Mancina RM, Dongiovanni P, Petta S, et al. The MBOAT7-TMC4 variant rs641738 increases risk of nonalcoholic fatty liver disease in individuals of European descent. Gastroenterology 2016;150:1219–1230 e6.

3. Pelusi S, Petta S, Rosso C, et al. Renin-angiotensin system inhibitors, type 2 diabetes and fibrosis progression: an observational study in patients with nonalcoholic fatty liver disease. PLoS One 2016;11:e0163069.
4. Colloredo G, Guido M, Sonzogni A, et al. Impact of liver biopsy size on histological evaluation of chronic viral hepatitis: the smaller the sample, the milder the disease. J Hepatol 2003;39:239–244.
5. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–1321.
6. Petta S, Valenti L, Marra F, et al. MERTK rs4374383 polymorphism affects the severity of fibrosis in non-alcoholic fatty liver disease. J Hepatol 2016; 64:682–690.



Supplementary Figure 1. Prevalence of nonalcoholic steatohepatitis (NASH) and lobular inflammation not fulfilling NASH criteria, vs simple steatosis according to fibrosis stage in patients stratified by (*A*) developmental vs adult age, (*B*) sex, and (*C*) *PNPLA3* I148M variant status (available in n = 1698).



Supplementary Figure 1. Continued.

Supplementary Table 1. Demographic, Anthropometric, and Clinical Features of the Cross-Sectional Liver Biopsy Cohort (n = 1738)

	Overall (n = 1738)	Liver clinic (n = 958; 55.1%)	Severe obesity (n = 780; 44.9%)	*P* value
Sex, female	860 (49.4)	299 (31.2)	561 (71.9)	<.0001
Age, *y*	43.7 ± 14.8	42.8 ± 17.5	44.7 ± 10.6	.99
BMI, *kg/m²*	34.6 ± 9.0	27.8 ± 4.0	42.9 ± 5.7	<.0001
Obesity	1047 (60.2)	267 (27.9)	780 (100)	<.0001
T2D/IFG, yes	466 (26.8)	228 (23.8)	238 (30.5)	.0017
Hypertension, yes	448 (33.3)	272 (29.3)	176 (42.5)	<.0001
Total cholesterol, *mg/dL*	191 ± 44	195 ± 44	185 ± 44	<.0001
Triglyceride level, *mg/dL*	139 ± 77	138 ± 80	140 ± 73	.55
HDL cholesterol, *mg/dL*	48 ± 14	48 ± 14	48 ± 14	.35
ALT, *IU/L*	42 (24–69)	54 (34–80)	30 (19–48)	<.0001
AST, *IU/L*	29 (21–43)	34 (24–47)	22 (17–33)	<.0001
NASH, yes	571 (32.9)	422 (44.0)	149 (19.2)	<.0001
Clinically significant fibrosis, stages F2–F4	389 (22.4)	287 (30.0)	102 (13.1)	<.0001
Severe fibrosis, stages F3–F4	174 (10.0)	137 (14.3)	37 (4.8)	<.0001
PNPLA3, 148M/M[a]	234 (13.8)	168 (17.7)	66 (8.8)	<.0001

NOTE. Values are reported as means ± SD, medians {interquartile range}, or number (%), as appropriate. Characteristics of participants were compared using a linear regression model or a logistic regression model (as required).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HDL, high-density lipoprotein; IFG, impaired fasting glucose; NASH, nonalcoholic steatohepatitis; T2D, type 2 diabetes.
[a]The *PNPLA3* I148M genotype was available in a subset of patients (n = 1698).

Supplementary Table 2. Histologic Activity in Patients Stratified by Fibrosis Stage

	Steatosis			Ballooning			Lobular inflammation			
Fibrosis stage	1	2	3	0	1	2	0	1	2	3
0	558 (74.9)	114 (15.3)	73 (9.8)	618 (82.9)	108 (14.5)	19 (2.6)	513 (68.9)	201 (27.0)	31 (4.2)	-
1	263 (44.4)	190 (31.6)	149 (24.7)	345 (57.3)	207 (34.4)	50 (8.3)	148 (24.6)	351 (58.3)	102 (16.9)	1 (0.2)
2	56 (26.0)	75 (34.9)	84 (39.1)	88 (40.9)	86 (40.0)	41 (19.1)	24 (11.2)	98 (45.6)	83 (38.6)	10 (4.6)
3	29 (27.1)	31 (29.0)	47 (43.9)	21 (19.6)	42 (32.2)	44 (40.2)	8 (7.5)	35 (32.7)	55 (51.4)	9 (8.4)
4	32 (47.8)	20 (29.8)	15 (22.4)	4 (6.0)	38 (56.7)	25 (37.3)	7 (10.4)	30 (44.8)	26 (38.8)	4 (6.0)

Supplementary Table 3. Independent Predictors of Clinically Significant Fibrosis (Stages F2–F4) in the Cross-Sectional LBC Patients Stratified According to the Presence of NASH and Modality of Recruitment (Liver Clinic Vs Severe Obesity)

	Liver clinic						Severe obesity					
	NASH			No NASH			NASH			No NASH		
	Estimate	SE	*P* value	Estimate	SE	*P* value	Estimate	SE	*P* value	Estimate	SE	*P* value
Sex, female	-0.092	0.139	.49	-0.073	0.157	.064	-0.314	0.223	.16	-0.461	0.163	.004
Age, *y*	+0.045	0.008	$1*10^{-8}$	+0.028	0.008	.001	+0.001	0.019	.96	+0.020	0.015	.18
BMI, kg/m^2	+0.098	0.034	.004	-0.015	0.037	.69	-0.054	0.047	.25	+0.054	0.024	.030
T2D/IFG, yes	+0.515	0.139	$2*10^{-4}$	+0.477	0.164	.003	+0.356	0.222	.10	+0.320	0.165	.053
Steatosis, grade	+0.072	0.146	.62	+0.409	0.152	.007	+0.147	0.255	.57	+0.821	0.175	$3*10^{-6}$
Ballooning, grade	+0.284	0.129	.028	+1.239	0.432	.005	+0.592	0.283	.037	+0.523	0.232	.024
Lobular inflammation, grade	+0.634	0.131	$1*10^{-6}$	+0.733	0.168	$1*10^{-5}$	+0.751	0.220	$7*10^{-4}$	+0.743	0.232	.001
PNPLA3, I148M alleles[a]	+0.078	0.163	.65	+0.437	0.196	.026	+0.359	0.183	.038	+0.497	0.239	.024

NOTE. Characteristics of participants were compared using linear regression models.
BMI, body mass index; IFG, impaired fasting glucose; LBC, liver biopsy cohort; NASH, nonalcoholic steatohepatitis; T2D, type 2 diabetes.
[a]The *PNPLA3* I148M genotype was available in a subset of patients (n = 1698).

Supplementary Table 4. Independent Predictors of Advanced Fibrosis (Stages F3–F4) in the Cross-Sectional LBC Patients Stratified According to the Presence of NASH

	NASH			No NASH		
	Estimate	SE	*P* value	Estimate	SE	*P* value
Sex, female	-0.142	0.118	.23	-0.350	0.214	.10
Age, *y*	+0.055	0.008	$3*10^{-10}$	+0.033	0.014	.022
BMI, kg/m^2	-0.037	0.018	.037	-0.025	0.022	.27
T2D/IFG, yes	+0.440	0.120	$2*10^{-4}$	+0.584	0.207	.005
Steatosis, grade	+0.150	0.134	.27	+0.395	0.217	.068
Ballooning, grade	+0.414	0.121	.007	+1.224	0.242	$4*10^{-7}$
Lobular inflammation, grade	+0.465	0.121	$1*10^{-4}$	+1.098	0.249	$1*10^{-5}$
PNPLA3, I148M alleles[a]	+0.333	0.160	.037	+0.563	0.280	.045

NOTE. Characteristics of participants were compared using linear regression models.
BMI, body mass index; IFG, impaired fasting glucose; LBC, liver biopsy cohort; NASH, nonalcoholic steatohepatitis; T2D, type 2 diabetes.
[a]The *PNPLA3* I148M genotype was available in a subset of patients (n = 1698).

Supplementary Table 5. Independent Predictors of Clinically Significant Fibrosis After Exclusion of Patients With Cirrhosis (Stages F2–F3) in Patients Stratified by the Presence of NASH

	NASH			No NASH		
	Estimate	SE	*P* value	Estimate	SE	*P* value
Sex, female	-0.144	0.113	.20	-0.362	0.116	.002
Age, *y*	+0.038	0.007	$8*10^{-8}$	+0.023	0.007	.002
BMI, kg/m^2	-0.022	0.015	.15	+0.005	0.012	.67
T2D/IFG, yes	+0.275	0.115	.017	+0.374	0.116	.001
Steatosis, grade	+0.147	0.129	.25	+0.569	0.114	$6*10^{-7}$
Ballooning, grade	+0.389	0.116	$8*10^{-4}$	+1.117	0.481	.02
Lobular inflammation, grade	+0.697	0.110	$2*10^{-10}$	+0.787	0.133	$4*10^{-9}$
PNPLA3, I148M alleles[a]	+0.101	0.143	.49	+0.432	0.153	.005

NOTE. Characteristics of participants were compared using linear regression models.
BMI, body mass index; IFG, impaired fasting glucose; NASH, nonalcoholic steatohepatitis; T2D, type 2 diabetes.
[a]The *PNPLA3* I148M genotype was available in a subset of patients (n = 1633).

Supplementary Table 6. Independent Predictors of Clinically Significant Fibrosis in Patients Without NASH Stratified by the Presence of Type 2 Diabetes

	T2D (n = 254)			No T2D (n = 911)		
	Estimate	SE	*P* value	Estimate	SE	*P* value
Sex, female	-0.188	0.183	.003	-0.412	0.145	.005
Age, *y*	+0.011	0.018	.32	+0.036	0.010	$3*10^{-4}$
BMI, kg/m^2	+0.002	0.019	.91	+0.011	0.015	.47
Steatosis, grade	+0.419	0.190	.026	+0.667	0.137	$1*10^{-6}$
Ballooning, grade	NA	NA	NA	+1.379	0.444	.002
Lobular inflammation, grade	+0.908	0.233	$1*10^{-4}$	+0.697	0.163	$2*10^{-5}$
PNPLA3, I148M alleles[a]	+0.354	0.254	.16	+0.519	0.185	.005

NOTE. Characteristics of participants were compared using linear regression models.
BMI, body mass index; NASH, nonalcoholic steatohepatitis; T2D, type 2 diabetes.
[a]The *PNPLA3* I148M genotype was available in a subset of patients (n = 1131).