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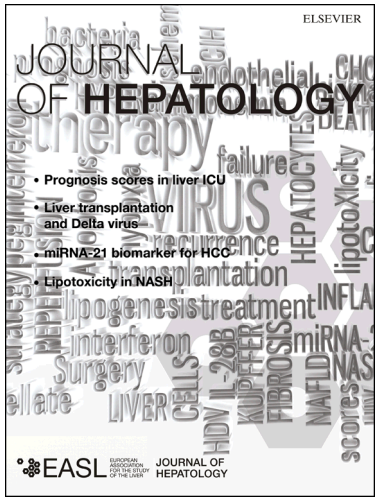

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Statin use and nonalcoholic steatohepatitis in at risk individuals

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ABBREVIATIONS

NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; HMGCR: 3-hydroxy-3-methyl-glutaryl coenzyme-A reductase; PNPLA3: Patatin-like phospholipase domain-containing 3; TM6SF2: Transmembrane 6 superfamily member 2; IFG: impaired fasting glucose; T2DM: type 2 diabetes mellitus; ALT: alanine aminotransferases; AST: aspartate aminotransferases; BMI: body mass index; SREBP1c: sterol regulatory element-binding protein 1c.

Keywords: cholesterol; NASH; nonalcoholic fatty liver disease; PNPLA3; nonalcoholic steatohepatitis; statin; steatosis.

CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest relevant for the present manuscript.

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PD: acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis;

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SR, JP: analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; obtained funding; study supervision

LV: study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; obtained funding; study supervision.

ABSTRACT

Background & Aims: Excess hepatic free cholesterol contributes to the pathogenesis of nonalcoholic steatohepatitis, and statins reduce cholesterol synthesis. Aim of this study was to assess whether statin use is associated with histological liver damage related to steatohepatitis.

Methods: The relationship between statin use, genetic risk factors, and liver damage was assessed in a multi-center cohort of 1,201 European individuals, who underwent liver biopsy for suspected nonalcoholic steatohepatitis.

Results: Statin use was recorded in 107 subjects, and was associated with protection from steatosis, NASH, and fibrosis stage F2-F4, in a dose-dependent manner (adjusted $p<0.05$ for all). In 100 treated patients matched 1:1 for modality of recruitment, gender, presence of impaired fasting glucose or type 2 diabetes, *PNPLA3* I148M risk alleles, *TM6SF2* E167K variant, age, and body mass index, statin use remained associated with protection from steatosis (OR 0.09, 95% c.i. 0.01-0.32; $p=0.004$), steatohepatitis (OR 0.25, 95% c.i. 0.13-0.47; $p<0.001$), and fibrosis stage F2-F4 (OR 0.42, 95% c.i. 0.20-0.8; $p=0.017$). Results were confirmed in a second analysis, where individuals were matched within recruitment center ($p<0.05$ for all). The protective effect of statins on steatohepatitis was stronger in subjects not carrying the I148M *PNPLA3* risk variant ($p=0.02$ for interaction), as statins were negatively associated with steatohepatitis in patients negative ($p<0.001$), but not in those positive for the I148M variant (p=NS).

Conclusions: Statin use was associated with protection towards the full spectrum of liver damage in individuals at risk of nonalcoholic steatohepatitis. However, the I148M *PNPLA3* risk variant limited this beneficial effect.

INTRODUCTION

Following the trail of the obesity epidemic, nonalcoholic fatty liver disease (NAFLD), defined by hepatic triglycerides content exceeding 5% in the absence of at risk alcohol intake [1], has become the leading cause of liver disease [2, 3]. This condition is epidemiologically associated with metabolic syndrome and insulin resistance, that increases the hepatic flux of free fatty acids from adipose tissue and leads to *de novo* lipogenesis [4-6].

NAFLD encompasses a spectrum of conditions ranging from simple steatosis, usually a non-progressive condition, to nonalcoholic steatohepatitis (NASH), characterized by hepatocellular damage, lobular necroinflammation, and fibrogenesis [7, 8]. NASH may evolve to cirrhosis and hepatocellular carcinoma [9, 10]. Although genetic variants influencing lipid droplets remodeling and lipoproteins secretion contribute to NAFLD susceptibility [11], the mechanisms underpinning liver disease progression are not fully understood. Furthermore, insulin sensitizing agents and antioxidants have so far provided disappointing results in the treatment of this condition [12].

NASH is characterized by increased hepatic free cholesterol accumulation, reflecting dietary factors and insulin resistance [13, 14], paralleled by increased expression and activity of 3-hydroxy-3-methyl-glutaryl coenzyme-A reductase (HMGCR), the rate-limiting enzyme in cholesterol biosynthesis [14-16]. Furthermore, in severely obese, hepatic cholesterol content correlates with liver damage severity [17]. Mitochondrial toxicity, endoplasmic reticulum stress, generation of toxic oxysterols and secondary effects on the metabolism of other lipids have been surmised to mediate the deleterious effect of cholesterol on NASH pathogenesis [14, 18].

Statins (HMGCR inhibitors) are widely used for prevention of cardiovascular events. Despite a mild to moderate elevation of aminotransferases is a common side effect during statin treatment, this is not associated with liver disease, so that in current guidelines

regular monitoring of liver enzymes is no longer recommended [19, 20]. Conversely, epidemiological studies have linked statin use with reduced risk of NAFLD, as detected by ultrasonography [21], and of hepatocellular carcinoma [22]. Statins have been tested in NAFLD treatment only in either underpowered, or not controlled, or in studies lacking histological assessment of liver damage, which still is the gold standard to assess the prognosis of the disease [23]. In these studies, statins were well tolerated and reduced cardiovascular risk [20, 24], but results on liver-related outcomes were not conclusive [25-30]. Furthermore, randomized trials testing the effect of statins in individuals with NASH would be difficult to design because these patients often require statin treatment to reduce cardiovascular risk [31, 32].

Primary aim of this study was to assess the relationship between statin use and liver damage, namely presence of steatosis, NASH, and of clinically significant fibrosis stage F2-F4, in a large multi-center cohort of patients (n=1,201) who underwent liver biopsy for suspected NASH [33]. As a secondary aim, we tested whether the association between statins and NASH was influenced by genetic risk factors.

PATIENTS AND METHODS

Patients

The association between statin use and liver damage was assessed in a previously described large multi-center cross-sectional cohort of individuals at risk of NASH [33]. In each individual, the specific statin molecule and dose were recorded. On the basis of the dose and potency of the specific drug, statin therapy was graded as low, moderate, or high-intensity according to current guidelines [32]. Clinical, metabolic, and genetic features (including evaluation of *Patatin-like phospholipase domain-containing 3* (*PNPLA3*) I148M and *Transmembrane 6 superfamily member 2* (*TM6SF2*) E167K risk variants) of these subjects have been reported in details [33]. Briefly, the cohort was made up of subjects of European descent from Italy and Finland, who underwent liver biopsy for suspected NASH due to increased liver enzymes associated with ultrasonographic evidence of steatosis and risk factors, or during routine examination at the time of bariatric surgery. Daily alcohol intake was <30/20 g in males/females, and coexistent causes of liver disease and uncontrolled hyperglycemia were ruled out.

Study design

The study design is presented in Figure S1. First, the clinical features of subjects on statin therapy, and the association between statin therapy with liver damage (presence of steatosis: grade 0 vs. 1-3, NASH, and clinically significant fibrosis F2-F4) were assessed in the whole cohort.

Next, to control for the different risk profile between treated and untreated patients, the impact of statin treatment on liver damage was evaluated by a nested case-control approach with careful matching of untreated patients (1:1 with treated ones), for indication

of liver biopsy (referral to hepatology clinic or evaluation during bariatric surgery), gender, presence of impaired fasting glucose (IFG) or type 2 diabetes mellitus (T2DM), *PNPLA3* I148M risk alleles, and presence of the *TM6SF2* E167K risk variant, age (±5 years), body mass index (BMI; ±2 Kg/m^2 for hepatology clinic patients, ±5 Kg/m^2 for bariatric surgery patients) (Match 1).

Finally, to control for possible confounding factors related to heterogeneous clinical management among recruiting centers and ethnicity, we conducted a further case-control analysis matching 1:1 treated with untreated patients with less stringent criteria for risk factors of liver damage, but with required matching within the single recruitment center (Milan bariatric, Kuopio bariatric, Milan hepatology, Palermo hepatology). Other matching criteria were presence of IFG/T2DM, gender, *PNPLA3* I148M/M genotype, presence of *TM6SF2* E167K variant, and age (±10 years) (Match 2).

If more than one untreated patients was available satisfying the matching criteria, the best possible match was chosen.

Ethical approval

The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki and the recommendations of the Ethics Committees of the Fondazione IRCCS Ca' Granda (Milan), Bambino Gesù Children's Hospital (Rome, Milan), Palermo University Hospital (Palermo), Northern Savo Hospital District in Kuopio (Finland), and University of Gothenburg (Sweden).

Histological evaluation

Clinically significant steatosis was defined as steatosis involving ≥5% of hepatocytes [7]. Diagnosis of NASH was based on the presence of steatosis with lobular necroinflammation

and ballooning or fibrosis. Disease activity was assessed according to the NAFLD activity score (NAS); fibrosis was staged according to the recommendations of the NAFLD clinical research network [7].

Statistical analysis

For descriptive statistics, continuous traits were summarized as means±SD. Two highly skewed variables—alanine aminotransferase (ALT) and aspartate aminotransferase (AST)—were summarized as medians and interquartile range. Categorical variables are shown as percentages. Analyses were performed by fitting data to generalized linear regression models. In particular, logistic regression models were fit to examine binary traits (presence of steatosis, NASH, clinically significant fibrosis stage F2-F4). Ordinal regression models were fit to examine semi-quantitative scores of histological liver damage (overall NAS, steatosis grade 0-3, ballooning plus necroinflammation grade 0-5, fibrosis stage 0-4). When specified, confounding factors were included in a model. ALT and AST levels were log-transformed before entry into the models.

In the analysis, the *TM6SF2* E172K variant was coded in a dominant genetic model because of its relatively low allele frequency [33]. The *PNPLA3* I148M variant was coded in an additive model [34].

Statistical analyses were carried out with JMP 11.0 (SAS Institute, Cary, NC) and SPSS 22.0 (IBM, Burbank, NJ). A two-sided P value <0.05 was considered statistically significant.

RESULTS

Characteristics of patients on statins

At the time of histological evaluation, a total of 107 individuals (9%) of the liver biopsy cohort were on statins for at least 6 months. Their clinical features in comparisons with those not on statins are shown in Table 1. Consistently with treatment indication for prevention of cardiovascular events, patients on statins were older, had higher BMI and prevalence of IFG/T2DM, and had more frequently been referred to a bariatric surgery service (all p values <0.001). Individuals on statins had lower fasting total and estimated LDL cholesterol levels (p<0.001), but higher circulating triglycerides (p=0.028). Of the treated patients, 52 (49%) were on simvastatin, 29 on rosuvastatin (27%), 18 atorvastatin (17%), 5 on pravastatin (4%), and 3 on fluvastatin (2%). Sixteen patients (15%) were on high-intensity, 78 (73%) on moderate-intensity, and 13 (12%) on low-intensity treatment.

Statin use and liver damage in the liver biopsy cohort

The impact of statin use on the severity of liver damage related to steatohepatitis (presence of steatosis, NASH, and fibrosis stage F2-F4) in the whole liver biopsy cohort is presented in Table 2. Cardiovascular risk factors affecting statin treatment indication such as age, male gender, BMI, IFG/T2DM, were generally independently associated with increased risk of liver damage (Table 2). Circulating triglycerides levels remained independently associated with steatosis and NASH. As expected, *PNPLA3* I148M risk alleles were consistently associated with the full spectrum of liver damage (p≤0.001).

After full adjustment for risk factors including study cohort (adult vs. pediatric), age, gender, BMI, presence of IFG or diabetes, *PNPLA3* I148M alleles, presence of the *TM6SF2* E167K variant, and circulating triglycerides, statin use was associated with reduced risk of

steatosis (OR 0.48, 95% c.i. 0.26-0.94; p=0.026), NASH (OR 0.62, 95% c.i. 0.40-0.97; p=0.037), and fibrosis stage F2-F4 (OR 0.59, 95% c.i. 0.34-0.98; p=0.041). Despite the aggregation of several risk factors for liver disease in individuals treated with statins, similar results were obtained after including subsequently all the confounding factors present in the fully adjusted model (Figure 1). There was no significant association between statin use and advanced fibrosis F3-F4 (OR 0.81, 95% c.i. 0.40-1.42; p=0.4).

After full adjustment for study cohort (adult vs. pediatric), age, gender, BMI, presence of IFG or diabetes, circulating triglycerides, *PNPLA3* I148M alleles, and presence of the *TM6SF2* E167K variant, (Figure 2), at ordinal regression analysis statin use was associated with protection from severity of NAFLD activity score (estimate -0.29, SE 0.10; p=0.006), steatosis severity (estimate -0.30, SE 0.10; p=0.003), a non-significant trend with disease activity (necroinflammation plus fibrosis; estimate -0.18, SE 0.10; p=0.061), and with severity of fibrosis (estimate -0.20, SE 0.10; p=0.047).

Sensitivity analyses

Because in the liver biopsy cohort were included 142 pediatric patients, for whom statins are not indicated, to exclude a selection bias we examined adults only (n=1,059). Statin use remained associated with presence of steatosis (OR 0.43, 95% c.i. 0.23-0.80; p=0.009), NASH (OR 0.63, 95% c.i. 0.40-0.97; p=0.035), and non-significantly associated with fibrosis stage F2-F4 (OR 0.62, 95% c.i. 0.33-1.00; p=0.050).

To exclude that clinical suspicion of advanced liver disease may have led to a selection bias in individuals treated with statins, we examined individuals without liver cirrhosis (n=1,154). Statin use remained associated with presence of steatosis (OR 0.31, 95% c.i. 0.17-0.57; p<0.001), and with NASH (OR 0.65, 95% c.i. 0.42-0.99; p=0.043). Association

with advanced fibrosis was not evaluated due to the exclusion of one extreme of the fibrosis distribution, namely individuals with cirrhosis.

Intensity of statin treatment and liver damage

In the overall cohort, no single statin molecule was significantly associated with liver damage severity ($p>0.05$). However, in the fully adjusted model we observed a trend for a dose-response effect of statin treatment intensity on the protection towards presence of steatosis (OR 0.73, 95% c.i. 0.54-1.00; $p=0.045$ for increasing treatment intensity from none to low, moderate, and high, shown in Figure S2), NASH (OR 0.79, 95% c.i. 0.64-0.99; $p=0.038$ for increasing treatment intensity, Figure S2), and fibrosis stage F2-F4 (OR 0.74, 95% c.i. 0.56-0.96; $p=0.028$ for increasing treatment intensity, Figure S2).

Statin use and liver damage in two nested case-control analyses

Since the association between statin use and liver damage in the whole cohort may be influenced by several confounders due to the higher risk profile of individuals treated with statins, the impact of statin use on liver damage was next analyzed in case-control analyses with two different matching criteria, namely Match 1 and Match 2.

Match 1

For Match 1 analysis, we found a suitable match for 100 treated patients (93%), 50 from hepatology clinics and 50 from bariatric services. The features of treated vs. untreated patients are shown in Table S1 and there were not significantly different, except that as expected total and LDL cholesterol levels, but not HDL and triglycerides, were altered in treated individuals ($p\leq0.002$).

The association between statin use and liver damage in this case-control analysis is shown in Table 3, upper panel. In line with the results from the whole cohort, statin use was associated with reduced risk of steatosis (p<0.001), NASH (p<0.001), and fibrosis F2-F4 (p=0.018). The association was maintained at multivariate analysis adjusted for recruitment criteria, age, sex, BMI, IFG/T2DM, *PNPLA3* I148M alleles and *TM6SF2* E167K variant: statin use was associated with reduced risk of steatosis (p=0.004), NASH (p<0.001), and fibrosis stage F2-F4 (p=0.017). At logistic regression analysis the association of statin use with NASH was also independent of the presence of steatosis (OR 0.37, 95% c.i. 0.19-0.70; p=0.002). Conversely, conditioning for NASH abolished the association between statin use and fibrosis stage F2-F4 (p>0.4).

At multivariate logistic regression analysis, there was no significant association between statin use and advanced fibrosis F3-F4 (OR 0.70, 95% c.i. 0.31-1.58; p=0.4).

Match 2

For Match 2 analysis, we found a suitable untreated match for 96 treated patients (90%). The features of treated patients and matched controls are shown in Table S2 and were not significantly different, except that as expected total and LDL cholesterol levels, but not HDL and triglycerides, were altered in treated patients (p≤0.009).

The association between statin use and liver damage in this case-control analysis is shown in Table 3, lower panel. In keeping with the results of the previous analysis, statin use was associated with reduced risk of steatosis (p=0.013), NASH (p<0.001), and fibrosis F2-F4 (p=0.047). The association was maintained at multivariate analysis adjusted for single recruitment center, age, sex, BMI, IFG/T2DM, *PNPLA3* I148M alleles and *TM6SF2* E167K variant, statin use was associated with reduced risk of steatosis (p=0.006), NASH (p<0.001), and fibrosis stage F2-F4 (p=0.016).

At multivariate logistic regression analysis, a significant association between statin use and reduced risk of advanced fibrosis F3-F4 was detected (OR 0.42, 95% c.i. 0.17-0.99; p=0.049).

Genetic determinants of liver disease and impact of statins on liver damage

Finally, we evaluated whether genetic risk variants influenced the association of statin use with NASH, the major study outcome.

The prevalence of NASH according to statin use in patients stratified by the I148M *PNPLA3* genotype in the Match 1 analysis is presented in Figure S3A. Statins were associated with lower prevalence of NASH in patients negative ($p<0.001$), but not in those positive for the I148M variant ($p=0.23$ and $p=0.68$ for 148I/M and 148M/M genotypes, respectively; $p=0.15$ in all patients positive for the 148M allele). Individuals on statins had lower prevalence of NASH irrespective of the *TM6SF2* E167K variant, but the association was not significant in patients positive for the mutation, possibly due to the low sample size (Figure S3B). Similar results were obtained in Match 2 analysis for *PNPLA3* I148M (Figure S3C), whereas in Match2 analysis statins showed a close trend for protection from NASH even in patients negative for E167K *TM6SF2* (Figure S3D; $p=0.06$ for lower prevalence of NASH in 167K positive subjects in statin users vs. non-users).

A significant interaction was detected in Match 1 analysis between statin use and *PNPLA3* genotype. Indeed, the product term (statin use * I148M alleles) was significant in the full multivariate model evaluating the risk of NASH, ($p=0.020$; Figure 3 and Figure S4). Conversely, *TM6SF2* did not influence the effect of statins on NASH (Figure S4). The interaction between statin use and *PNPLA3* genotype on the risk of NASH was confirmed in Match 2 analysis (Figure 3; $p=0.038$).

Among the other variables, modality of enrolment (bariatric surgery vs. hepatology service) influenced the association of statins with NASH in the Match 1 analysis (p=0.010; Figure S3), but this was not confirmed in the Match 2 analysis.

DISCUSSION

The main finding of this study is that statin use was associated with protection against the full spectrum of liver damage related to NAFLD, namely presence of steatosis, NASH, and fibrosis stage F2-F4 in a large cross-sectional cohort of 1,201 at risk individuals. Supporting a causal link between exposure to statins and protection from hepatic disease, there was a dose-dependent relationship between intensity of statin treatment (from none, to low, moderate and high) and reduced risk of histological lesions. The independent association between statin use and liver disease protection was confirmed in an even more clear fashion after careful matching treated with untreated patients for modality of referral, demographic, metabolic, and genetic risk factors. Indeed, consistently with treatment indication for prevention of cardiovascular events, patients on statins were older, had higher BMI and prevalence of IFG/T2DM, and had higher circulating triglycerides, likely reflecting more severe dyslipidemia prior to treatment [35].

The study did not include patients with clinical evidence of cirrhosis, and exclusion of subjects with histological cirrhosis did not abolish the association of statin use with steatosis and NASH. These data suggest that the association between statins and protection from liver damage was not biased by a lower likelihood of prescription of these drugs to patients with more severe noninvasive biomarkers of liver damage (e.g. lower platelets or albumin levels) because of the clinical suspicion of advanced liver fibrosis.

The liver biopsy cohort is composed by a series of cases enrolled in five different hepatology and bariatric surgery services in Italy and Finland. An additional case-control analysis with less stringent, but enrolment center-restricted matching, fully confirmed the protective association between statins and liver damage. This evidence thereby suggests that the hepatoprotective effect of statins was not biased by ethnicity, unmeasured environmental factors, and different clinical management among the centers of the cohort.

In statin treated patients, steatosis was reduced by 48-91%, with the greater effect observed after careful matching of patients. Liver of patients treated with statins down-regulates the sterol regulatory element-binding protein 1c (SREBP1c), a key regulator of triglycerides synthesis, as well as other genes involved in lipid synthesis [30]. It could therefore be speculated that besides inhibiting cholesterol synthesis, statins indirectly regulate triglycerides synthesis via inhibition of SREBP1c.

Furthermore, in statin treated patients, NASH decreased by 38-76% and significant fibrosis was almost halved. In matched patients the association of statin use with protection from NASH was independent of steatosis, but conditioning for NASH abolished the link between statin use and fibrosis. Therefore, statins may directly impact on NASH either by decreasing intracellular free cholesterol levels or by exerting a direct anti-inflammatory activity. Conversely, protection from fibrosis could be secondary to reduced inflammation. However, we cannot exclude that an independent anti-fibrotic effect of statins could be detected in larger cohorts [36]. Experimental studies are required to test the effect and mechanisms liking statins with hepatic lipogenesis and inflammation.

Next, we examined the effect of genetic variant predisposing to NASH and involved in hepatic lipid remodeling and lipoprotein secretion. Interestingly, in matched treated vs. untreated patients, the protective effect of statins on liver damage was blunted by the 148M *PNPLA3* risk allele, the major common genetic determinant of NASH [37]. These data are consistent with evidence indicating that *PNPLA3* I148M predisposes to NASH by inhibiting lipid efflux from hepatocellular lipid droplets [38-40], and that there is a dissociation between *de novo* lipogenesis and severity of hepatic steatosis in carriers of the I148M variant [41]. Furthermore, the reduction in the protection of statins on NASH in carriers of the *PNPLA3* risk allele may be due to a direct involvement of the I148M variant in inflammation and fibrosis mediated by its role on retinol metabolism in hepatic stellate cells

[42]. It is worth of note that the *PNPLA3* I148M variant reduces also the beneficial anti-lipogenic effect of omega-3 fatty acids in patients with NAFLD [43].

Our data suggest that long-term use of statins protect from liver damage in subjects with NAFLD or severe obesity. Adequately powered randomized controlled trials would be required to formally test this hypothesis, although these studies would be difficult to design because of the indication of statins in a large fraction of individuals with NASH for prevention of cardiovascular events. Stratification for *PNPLA3* I148M genotype may play an important role to get reliable results. Meanwhile, statin use should be encouraged for cardiovascular events prevention in individuals with NASH [24].

A limitation of this study is that, due to the cross-sectional retrospective design, we cannot infer any definite causal relationship between drug exposure and liver related outcomes. Since treatment information was collected at the time of liver biopsy, and data on initiation of pharmacological treatments older than 6 months were not available, we could not assess the relationship between the minimal duration of statin exposure required to detect a protective effect on liver damage. Furthermore, possibly due to the relatively low proportion of individuals with advanced fibrosis included in the present cohort, we could not consistently detect a protective effect of statins on fibrosis stage F3-F4, which should be evaluated in future studies.

Finally, we cannot exclude that, although benefits generally far outweigh the risks, statins may induce hepatotoxicity and liver-unrelated adverse events in a small proportion of individuals at risk of NASH [44]. In particular, due to the study design, we cannot exclude that statins may have contribute to diabetes development in some treated individuals [45]. However, our findings are in line with previous evidence indicating that statins are generally well tolerated in patients with NAFLD, and may improve liver-related outcomes modulating the metabolism of both cholesterol and triglycerides [13-17, 25-30].

Finally, in the present cohort reflecting clinical practice in tertiary centers, statins seemed to be under-prescribed, since only 10% of adults in this high-risk population were treated. Our study shows that fear from liver injury is no reason to withhold statin treatment. On the contrary, this is a group of patients with increased risk for cardiovascular disease that often will benefit from statin treatment [46].

In conclusion, our data suggest that statin use associates with protection towards the full spectrum of liver damage in a dose-dependent manner in a large cross-sectional cohort of patients at risk of NASH. However, presence of the I148M *PNPLA3* risk variant reduced this beneficial effect. In the absence of randomized controlled trials, more observational studies should be performed to clarify the potential role of statins in the prevention of liver disease related to NASH.

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TABLES

Table 1. Demographic, anthropometric and clinical characteristics of the liver biopsy cross-sectional cohort (n=1,201) stratified by statin therapy.

	Statin therapy		
	No	Yes	
Characteristic	n=1,094 (89%)	n=107 (9%)	P value
Age, years	41±16	53±10	<0.001
Sex, female	516 (47)	59 (55)	0.13
Enrolment,	501/451/142	50/57/0	
Hepatology/bariatric/pediatric	(46/41/13)	(47/53/0)	<0.001
BMI, kg/m^2	34.0±9	36.2±9	<0.001
IFG/T2DM	270 (25)	56 (52)	<0.001
Total cholesterol, mmol/l	4.6±1.1	4.0±1.3	<0.001
LDL cholesterol, mmol/l	3.1±1.0	2.5±1.2	<0.001
HDL cholesterol, mmol/l	1.2±0.4	1.1±0.4	0.060
Triglycerides, mmol/l	1.6±0.9	1.8±0.8	0.028
ALT, IU/l	45 {25-72}	42 {29-59}	0.30
AST, IU/l	30 {21-44}	30 {21-41}	0.52
PNPLA3, 148I/I	488 (45)	52 (49)	0.48
148I/M	454 (41)	38 (35)	
148M/M	152 (14)	17 (16)	
TM6SF2, E167K	146 (13)	11 (10)	0.45

Values are mean ± SD, or number (%).

BMI: body mass index; IFG: impaired fasting glucose; T2DM: type 2 diabetes mellitus.

Table 2. Independent predictors of liver damage (presence of steatosis, NASH, and fibrosis stage F2-F4) in 1,201 patients of the liver biopsy cross-sectional cohort.

	Steatosis			NASH			Fibrosis F2-F4		
	OR	95% c.i.	P	OR	95% c.i.	P	OR	95% c.i.	P
Age, years	1.01	0.99-1.02	0.19	1.01	1.00-1.02	0.017	1.02	1.01-1.04	<0.001
Sex, F	0.61	0.37-1.00	0.048	0.84	0.64-1.11	0.22	0.74	0.52-1.00	0.043
BMI, Kg/m2	1.07	1.04-1.10	<0.001	1.01	0.99-1.03	0.13	0.98	0.95-0.99	0.039
IFG/T2DM, yes	1.34	0.80-2.31	0.27	2.13	1.57-2.90	<0.001	2.13	1.57-2.90	<0.001
Triglycerides, mM	1.57	1.16-2.22	0.006	1.26	1.08-1.48	0.004	1.02	0.85-1.21	0.83
PNPLA3, I148M alleles	1.80	1.27-2.61	0.001	1.75	1.45-2.11	<0.001	1.55	1.26-1.91	<0.001
TM6SF2, E167K	1.43	0.71-3.21	0.35	1.82	1.23-2.72	0.002	1.44	0.97-2.20	0.068
Statin, yes	0.48	0.26-0.94	0.026	0.62	0.40-0.97	0.037	0.59	0.34-0.98	0.041

OR: odds ratio; c.i.: confidence interval; BMI: body mass index, IFG: impaired fasting glucose; T2DM: type 2 diabetes mellitus.

Comparisons were made by fitting data to logistic regression models. P values were adjusted for age, gender, BMI, IFG/T2DM, serum triglycerides, *PNPLA3* I148M alleles, presence of the *TM6SF2* E167K variant, and study cohort.

Table 3. Statin use and liver damage severity (presence of steatosis, NASH, fibrosis stage F2-F4) in Match 1 (upper panel) and Match 2 (lower panel) analyses.

Match 1°	Statin therapy		Unadjusted			Adjusted*		
	Yes (n=100)	No (n=100)	OR	95% c.i.	p value	OR	95% c.i.	p value
Steatosis, yes	83 (83)	98 (98)	0.10	0.02-0.36	<0.001	0.09	0.01-0.32	0.004
NASH, yes	47 (47)	77 (77)	0.26	0.14-0.48	<0.001	0.25	0.13-0.47	<0.001
Fibrosis, F2-F4	23 (23)	38 (38)	0.48	0.26-0.88	0.018	0.42	0.20-0.85	0.017
Match 2^	Statin therapy		Unadjusted			Adjusted*		
	Yes (n=96)	No (n=96)	OR	95% c.i.	p value	OR	95% c.i.	p value
Steatosis, yes	79 (82)	90 (94)	0.31	0.11-0.78	0.013	0.23	0.07-0.67	0.006
NASH, yes	43 (44)	73 (76)	0.24	0.13-0.45	<0.001	0.20	0.10-0.39	<0.001
Fibrosis, F2-F4	19 (20)	31 (32)	0.52	0.26-0.99	0.037	0.37	0.16-0.87	0.016

OR: odds ratio; 95% c.i.: 95% confidence interval; NASH: nonalcoholic steatohepatitis.

Prevalence data are shown as number (% value). Comparisons were made by fitting data to logistic regression models. * Adjusted for recruitment criteria, age, sex, BMI, presence of IFG/T2DM, *PNPLA3* I148M alleles, presence of *TM6SF2* E167K variant.

° Match 1 analysis is a group of 100 individuals on statin treatment matched with 100 individuals from those untreated based on the following criteria: recruitment criteria (bariatric surgery vs. hepatology service), gender, presence of IFG/T2DM, *PNPLA3* I148M alleles, presence of *TM6SF2* E167K variant, age, and BMI.

^ Match 2 analysis is a group of 96 individuals on statin treatment matched with 96 individuals from those untreated based on the following criteria: recruitment center, gender, presence of IFG/T2DM, *PNPLA3* 148M/M genotype, presence of *TM6SF2* E167K variant, and age.

FIGURE LEGENDS

Figure 1. Liver damage risk (presence of steatosis, NASH, and fibrosis stage F2-F4) conferred by statin use in 1,201 patients of the liver biopsy cross-sectional cohort. Comparisons were made by fitting data to logistic regression models. OR and 95% confidence intervals of liver damage for statin use were adjusted for variables specified on the left ("+" indicated that new independent variables specified were sequentially added to the precedent model).

Figure 2. Grading of liver damage (NAFLD activity score, steatosis grade, disease activity, that is ballooning plus necroinflammation, and fibrosis stage) according to statin use in 1,201 patients of the liver biopsy cross-sectional cohort. Comparisons were made by fitting data to ordinal regression models adjusted for study cohort, age, gender, BMI, presence of IFG or diabetes, circulating triglycerides, *PNPLA3* I148M alleles, and presence of the *TM6SF2* E167K variant.

Figure 3. Association of statin use with the risk of NASH in treated and matched untreated patients by PNPLA3 *I148M* genotype in the Match 1 and Match 2 analyses. Odds ratios and 95% confidence intervals of NASH for statin use were estimated at logistic regression analysis adjusted for age, BMI, modality of enrolment (bariatric surgery vs. hepatology service), gender, presence of IFG/T2DM, and presence of *TM6SF2* E167K variant. P values were calculated by testing the interaction term statin use * I148M alleles in the full model.

Figure 1

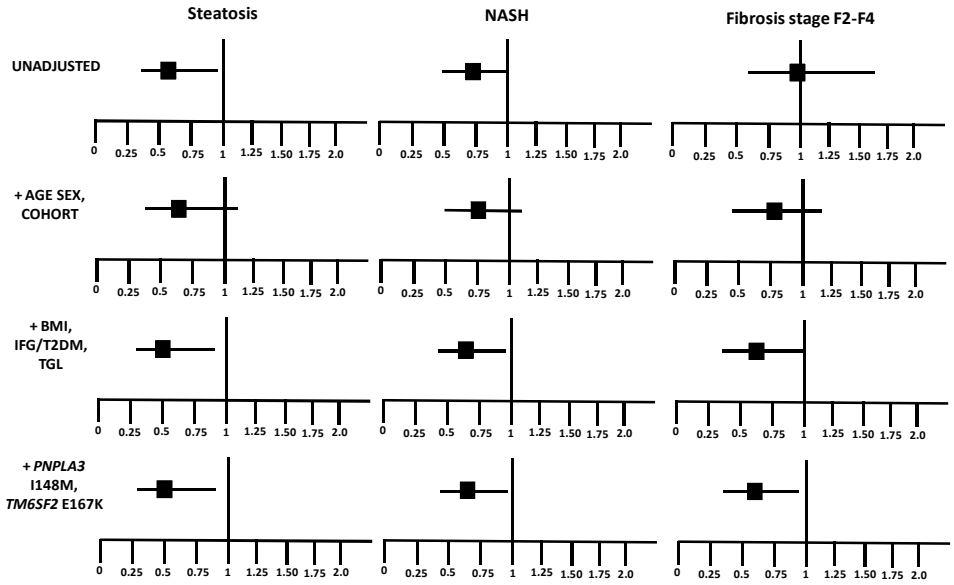

Fig. 1

Figure 2

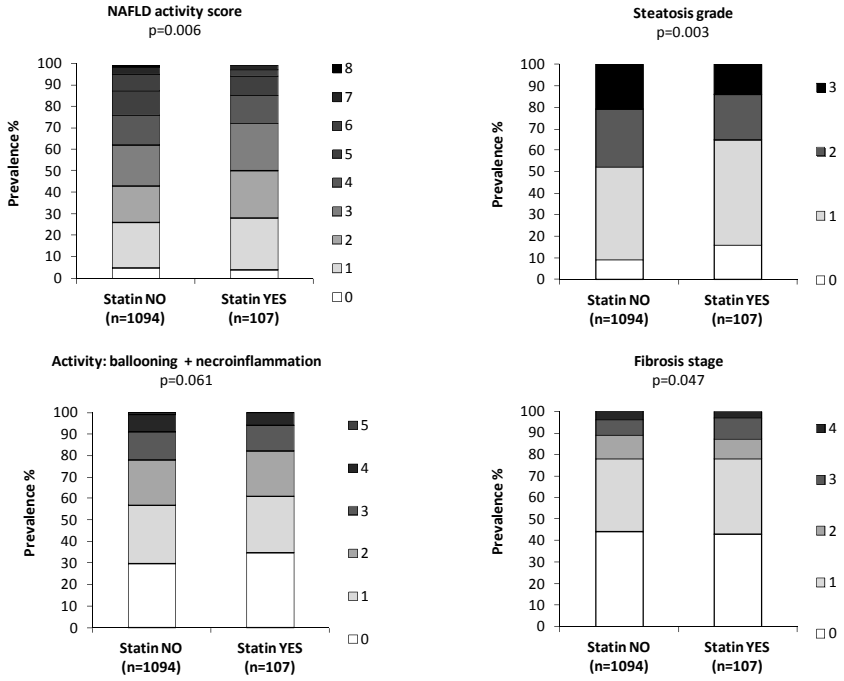

Figure 3