The Protection Conferred by *HSD17B13* rs72613567 Polymorphism on Risk of Steatohepatitis and Fibrosis May Be Limited to Selected Subgroups of Patients With NAFLD

Eduardo Vilar-Gomez, MD, PhD[1], Carlos J. Pirola, PhD[2], Silvia Sookoian, MD, PhD[3], Laura A. Wilson, ScM[4], Tiebing Liang, PhD[1] and Naga Chalasani, MD[1]

INTRODUCTION: **Our study aimed to explore how *PNPLA3* rs738409 or phenotypic risk factors may moderate the relationship between *HSD17B13* rs72613567 and risk of steatohepatitis and fibrosis.**

METHODS: **This analysis consisted of 1,153 non-Hispanic whites with biopsy-proven nonalcoholic fatty liver disease enrolled in the nonalcoholic steatohepatitis Clinical Research Network studies. Nonalcoholic fatty liver disease severity was determined by liver histology scored centrally according to the nonalcoholic steatohepatitis Clinical Research Network criteria. Moderation and logistic regression analyses were performed to identify the influence of moderators (*PNPLA3* rs738409, age, sex, body mass index, and diabetes) on the relationship between *HSD17B13* rs72613567 and risk of steatohepatitis and fibrosis.**

RESULTS: ***HSD17B13* rs72613567 genotype frequency was as follows: (−/−), 64%; (−/A), 30%; (A/A), 6%. Moderation analysis showed that the protective effect of *HSD17B13* rs72613567 A-allele on risk of steatohepatitis remained only significant among patients with *PNPLA3* rs738409 genotype CC (β coeff: −0.19, *P* = *0*.019), women (β coeff: −0.18, *P* < 0.001), patients of age ≥ 45 years (β coeff: −0.18, *P* < 0.001), patients with body mass index ≥ 35 kg/m² (β coeff: −0.17, *P* < 0.001), and patients with diabetes (β coeff: −0.18, *P* = *0*.020). Among women, the protective effect of *HSD17B131* rs72613567 A-allele on risk of steatohepatitis was stronger in those aged ≥ 51 years. Logistic regression-based sensitivity analysis including various important subgroups confirmed our observations.**

DISCUSSION: **The protection conferred by *HSD17B13* rs72613567 A-allele on risk of steatohepatitis and fibrosis may be limited to selected subgroups of individuals who are aged ≥ 45 years, women and have class ≥ 2 obesity or diabetes, and those with *PNPLA3* rs738409 CC genotype.**

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/A669, http://links.lww.com/CTG/A670, http://links.lww.com/CTG/A671, http://links.lww.com/CTG/A672, http://links.lww.com/CTG/A673, http://links.lww.com/CTG/A674, http://links.lww.com/CTG/A675

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a multifactorial disease in which genetic risk factors and associated comorbidities might play key roles in the development and the progression of this disease. Recent genome-wide and exome-wide association studies have identified 2 important variants—rs738409 in *PNPLA3* (1) and rs72613567 in *HSD17B13* (2)—linked to NAFLD and its progression. In 2018, Abul-Husn et al. (3) reported that a loss-of-function splice variant (rs72613567) of the *HSD17B13* gene was significantly associated with a lower risk of NAFLD progression and chronic liver disease. Furthermore, the protective effect of the *HSD17B13* rs72613567 A-allele against NAFLD, alcoholic liver

[1]Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana, USA; [2]Molecular Genetics and Biology of Complex Diseases, University of Buenos Aires-National Scientific and Technical Research Council (CONICET), Ciudad Autonoma de Buenos Aires, Argentina; [3]Department of Clinical and Molecular Hepatology, Institute of Medical Research (IDIM), University of Buenos Aires-National Scientific and Technical Research Council (CONICET), Ciudad Autonoma de Buenos Aires, Argentina; [4]Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA.
Correspondence: Naga Chalasani, MD. E-mail: nchalasa@iu.edu.
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disease, and hepatocellular carcinoma has been extensively confirmed in other studies including more diverse and heterogeneous populations (2,4–10).

HSD17B13 encodes for a liver-specific lipid droplet (LD)–associated protein (11) that seems to be implicated in hepatic-lipid biogenesis and metabolism. *HSD17B13* expression is significantly upregulated in the liver of patients and mice with NAFLD (7,12). Most recently, 17β-HSD13 has been reported to regulate retinoid metabolism by acting as a retinol dehydrogenase (7). 17β-HSD13 has been grouped with the short-chain dehydrogenases/reductases family, of which some members play a key role in the final step of estrogen and androgen steroid metabolism (13). However, the role of 17β-HSD13 in the regulation of sex steroid metabolism is still unclear and remains to be elucidated (14).

PNPLA3 rs738409 is another genetic variant strongly implicated in the regulation of lipid metabolism and LD biology (1). The *PNPLA3* rs738409 minor allele (G) is recognized as a major determinant in the progression of NAFLD to steatohepatitis, cirrhosis, and even hepatocellular carcinoma (15,16). PNPLA3 has a triglyceride hydrolase activity and acts as a lipase responsible for retinyl palmitate hydrolysis in hepatic stellate cells (HSCs) in humans. In the presence of *PNPLA3*-rs738409 variation, there is reduced activity of these enzymatic activities, leading to impairment of lipid catabolism, LDs remodeling, and very-low density lipoprotein secretion in hepatocytes with reduced fatty acid hydrolysis, increased triglyceride accumulation, and decreased very-low density lipoprotein secretion; all these events result in hepatic steatosis, inflammation, and fibrosis (1,17,18).

Little is known about the interplays between *PNPLA3* and *HSD17B13* and underlying clinical conditions and their impact on the development and progression of NAFLD. The knowledge of the biological interactions between genetic variants with opposite effects on the disease risk, including *PNPLA3* rs738409 that confers about 3.2-fold greater risk per G-risk allele of inflammation and fibrosis (15) and *HSD17B13* rs72613567 that associates with approximately 40% reduction in the odds of developing steatohepatitis per A-INS allele (2), may increase the predictive accuracy of genetic risk assessment. Similarly, understanding the impact of these interactions on clinical phenotypes is a critical step to identity patients at high risk of NAFLD progression in whom more precise and targeted therapies could halt the disease progression. Thus, our study was designed to explore how *PNPLA3* rs738409 and associated conditions might modify or moderate the relationship between *HSD17B13* rs72613567 and risk of steatohepatitis and fibrosis. To this end, we assessed gene–gene and gene–phenotype interactions.

MATERIAL AND METHODS

Study design and participants

This cross-sectional analysis comprised patients enrolled in the nonalcoholic steatohepatitis Clinical Research Network (NASH CRN) studies, including NAFLD Adult Databases 1 and 2 (*NCT01030484*), along with PIVENS (*NCT00063622*) and FLINT (*NCT01265498*) trials. Detailed information on patients' characteristics, including demographics, laboratory, and liver histology of NASH CRN studies, has been previously published (19–21). Some participants included in this analysis were part of the study by Ma et al. that investigated the association between *HSD17B13* variants and liver histology in patients with NAFLD (7). In brief, NASH CRN is a multiethnic prospective cohort recruiting patients with biopsy-proven NAFLD from 9 clinical centers across the United States. Appropriate institutional review boards approved the NASH CRN study, and all participants provided written informed consent. Participants were 18 years or older and had a histological diagnosis of NAFLD (≥5% of hepatocytes containing macrovesicular fat) in absence of a significant history of alcohol intake (>14 or >21 standard drinks for women and men, respectively) in the previous 2 years of the study enrollment.

A total of 1,697 adults with histologically confirmed NAFLD were enrolled through December 2019. Of them, 1,153 (68%) non-Hispanic whites with detailed information for *HSD17B13* rs72613567, *PNPLA3* rs738409, alcohol intake, centrally scored liver histology severity, and clinical phenotypes were included. All laboratory, clinical, and histological data analyzed in this study were obtained within 6 months of the liver biopsy. A thorough description of our selected cohort of non-Hispanic whites has been previously published (22).

Clinical and laboratory assessment

Baseline examinations included a medical history interview, physical examination, and blood draw. Body mass index (BMI) was calculated as body weight in kilograms divided by height in square meters. Diabetes was defined as a fasting plasma glucose level ≥ 126 mg/dL, 2-hour oral glucose tolerance test glucose of ≥ 200 mg/dL, glycosylated hemoglobin level of 6.5% or higher, or current use of glucose-lowering therapy. Alcohol consumption was obtained through Alcohol Use Disorders Identification Test (23) and Lifetime Drinking History (24) questionnaires. Nonheavy drinking was classified as a history of alcohol intake ≤14 and ≤21 standard drinks per week for women and men, respectively, during the 2 years preceding study enrollment. Nondrinkers were those who did not report alcohol intake at any time or those who had a history of nonheavy alcohol intake but became abstainers in the last 2 years before study enrollment. Other covariates including lipid-related panel, alanine (ALT) and aspartate (AST) aminotransferases, and a history of tobacco use were also gathered for study purposes.

Histologic evaluations

As per protocol, all liver biopsies are reviewed in a blinded manner by the NASH CRN Pathology Committee and scored according to the NASH CRN Scoring System (25) as follows: steatosis (0–3), lobular inflammation (0–2), ballooning degeneration (0–2), and overall fibrosis (0–4). The diagnosis of steatohepatitis was based on the severity of individual lesions and their patterns of distribution and classified as follows: not steatohepatitis, borderline steatohepatitis, and definite steatohepatitis (26). For study purposes, clinically significant fibrosis was considered as the presence of a stage of fibrosis ≥2, whereas a diagnosis of steatohepatitis was established in the presence of either borderline or definite steatohepatitis. Our primary study outcomes were risk of fibrosis and steatohepatitis.

Genetic variants, genotyping, and quality control

We analyzed *HSD17B13* rs72613567 and *PNPLA3* rs738409 genetic variants because of their established association with liver histology severity. Some of the data used in our analysis were partly reported in earlier articles on the discovery of susceptibility genotypes (7,27,28). The DNA was prepared from blood drawn at baseline; *HSD17B13* rs72613567 and *PNPLA3* rs738409 were successfully genotyped in 100% of the DNA samples. All DNA samples were blindly duplicated to assess the reproducibility of

genotypes. A reproducibility of 100% was obtained. Detailed information on rs738409 and rs72613567 genotyping can be found in the Supplementary Material (see Supplementary Digital Content 1, http://links.lww.com/CTG/A669).

Statistical analysis

Categorical variables were expressed as frequencies/percentages and compared by χ^2 test or Cochran-Armitage trend test for ordered alternatives. Continuous variables were presented as mean ± SD and compared through *t* test or Mann-Whitney *U* test, depending on whether they were or not normally distributed, respectively. The one-way analysis of variance was used to determine whether there were any statistically significant differences between the means of *HSD17B13* rs72613567 genotypes.

Either ordinal or binary logistic regression models were implemented to assess the association of genetic variants or underlying risk factors with histologic outcomes including 2 or more ordered categories, respectively. For genotypes, logistic regression models assumed either an additive (−/−), (−/A), (A/A) coded 0, 1, or 2, respectively, or a dominant (−/−) or (−/A) + (A/A) genetic model. Odds ratios (OR) and their confidence intervals (CIs) express the strength of the association of genetic variants or potential risk factors with histologic outcomes. All analyses were adjusted for well-known risk factors associated with NAFLD severity: age, sex, type 2 diabetes (T2D), BMI, *PNPLA3* rs738409, and nonheavy alcohol intake.

We further investigated the effect of interactions of *HSD17B13* rs72613567 with either *PNPLA3* rs738409 or selected risk factors and its impact on histologic outcomes. First, we created multiplicative interaction terms between *HSD17B13* rs72613567 and each risk factor (*PNPLA3* rs738409, age, BMI, sex, and T2D) using logistic regression models and including significant fibrosis or steatohepatitis as binary outcomes. Second, we used the margins command to visualize and interpret adjusted predictions of histologic outcomes for each *HSD17B13* rs72613567 genotype at different levels of each risk factor (29). Third, we performed a moderation analysis that examined whether our selected risk factors can strengthen, weaken, or reverse the nature of the relationship between *HSD17B13* rs72613567 and histologic outcomes (30). Moderation analyses were performed in SPSS statistical software using model 1 of *PROCESS* macro (version 3.5), which includes *HSD17B13* rs72613567 as the exposure variable, each risk factor as a moderator, and histologic scores as individual outcomes (31). Conditional effects (β coefficients) were determined using 95% bootstrap bias-corrected CIs based on 10,000 bootstrap samples (32,33). β coefficient weights provide an index of the magnitude of the effect size (31) between *HSD17B13* rs72613567 and histologic outcomes, and it is considered significant if the upper and lower bounds of the 95% bias-corrected CIs do not contain zero (31). Moderation analyses were adjusted for age, sex, T2D, BMI, *PNPLA3* rs738409, and nonheavy alcohol intake. Moderation analyses were initially performed under an additive genetic model of *HSD17B13* rs72613567. Results based on this genetic model displayed a dose-response relationship between (−/A) and (A/A) and the risk of NAFLD severity. Thus, we also decided to examine the effect of *HSD17B13* rs71623567 on histologic outcomes under a dominant genetic model. Further details are included in the Supplementary Material, (see Supplementary Digital Content 1, http://links.lww.com/CTG/A669), including conceptual diagrams of our moderation models.

Because moderation analyses showed that the effect of *HSD17B13* rs72613567 on the risk of NAFLD severity was markedly different at selected strata of moderators, sensitivity analyses were conducted separately for men and women, diabetes (yes or no), BMI (<35 or ≥35), age (<45 or ≥45), and *PNPLA3* rs738409 (CC or GC + GG), and all multivariable analysis were adjusted for nonheavy alcohol intake along with those covariates who were not tested as moderators itself. The cutoff points for age and BMI were selected through margins analysis, which showed that the risk of having steatohepatitis among individuals with *HSD17B13* rs72613567 (−/−) or (−/A) + (A/A) genotypes were significantly different starting at a BMI of ≥35 ($P = 0.009$) or an age of 45 years or older ($P = 0.048$).

Because our data showed that *HSD17B13* rs72613567 might have opposite effects regarding steatosis and fibrosis severity, we sought to investigate whether the effect of this variant on inflammation and fibrosis scores might be explained partly by a change in steatosis severity. To do so, a causal mediation analysis was performed through the *CAUSALMED* procedure (SAS Institute) (34). This is a regression-based approach that examines whether an explanatory variable (i.e., *HSD17B13* rs72613567) exerts its effect on outcome (i.e., fibrosis severity) through direct (*HSD17B13* rs72613567 itself) and/or indirect (i.e., steatosis) pathways. A more detailed explanation of causal mediation analysis can be found in the Supplementary Material (see Supplementary Digital Content 1, http://links.lww.com/CTG/A669).

To adjust for multiple testing, false discovery rate correction was performed using Benjamini-Hochberg procedure, and adjusted 95% CI of the estimates and Q values were calculated through *qqvalue* package (Stata software), with a $q < 0.05$ considered statistically significant (35,36). Statistical analyses were performed with SAS version 9.4 (SAS Institute, Cary, NC), STATA SE version 16 (Stata, College Station, TX), or SPSS version 26 (Chicago, IL).

RESULTS

Baseline characteristics of the cohort based on *HSD17B13* rs72613567 genotypes

A total of 1,153 non-Hispanic whites with biopsy-proven NAFLD were included after fulfilling our inclusion and exclusion criteria (see Supplemental Figure 1, Supplementary Digital Content 2, http://links.lww.com/CTG/A670). Among non-Hispanic whites, the genotype frequency of *HSD17B13* rs72613567 was as follows: (−/−) (0.64), (−/A) (0.30), and (A/A) (0.6), whereas the minor allelic frequency (A-insertion) was 0.21. Associations between *HSD17B13* rs72613567 genotypes and baseline characteristics are summarized in Table 1. Clinical, demographic, anthropometric, and *PNPLA3* rs738409 features were evenly distributed across all *HSD17B13* rs72613567 genotypes. Under an additive genetic model, the strength and the direction of the associations of *HSD17B13* rs72613567 with aminotransferases levels and histologic scores severity was consistent with published data (2,3,6,7).

Adopting an additive genetic model of *HSD17B13* rs72613567, the A-insertion allele was associated with increased risk of steatosis (OR: 1.35, $P < 0.001$) but decreased risks of lobular inflammation (OR: 0.69, $P < 0.001$), ballooning (OR: 0.80, $P = 0.022$), steatohepatitis (OR: 0.77, $P = 0.010$), and fibrosis (OR: 0.84, $P = 0.050$) (Table 2). Adopting a dominant genetic model, which includes 1 or 2 copies of allele A and it is justified by the low number of homozygous A/A, the associations of *HSD17B13* rs72613567 with

Table 1. Baseline characteristics according to *HSD17B13* rs72613567 genotypes

Variables	(−/−) n = 737	(−/A) n = 348	(A/A) n = 68	*P* value
Age (yr)	51.13 ± 11.32	50.02 ± 11.74	50.01 ± 11.57	0.283
Sex (women), n (%)	468 (64)	213 (61)	46 (68)	0.986
Type 2 diabetes mellitus, n (%)	265 (36)	114 (33)	29 (43)	0.940
Hypertension, n (%)	446 (61)	198 (57)	41 (60)	0.452
Tobacco use, n (%)	62 (9)	22 (6)	5 (8)	0.330
Body mass index (kg/m^2)	34.77 ± 6.45	34.87 ± 6.19	35.88 ± 7.14	0.399
Nonheavy drinkers, n (%)	277 (38)	134 (38)	22 (32)	0.704
PNPLA3 rs738409 (GC) + (GG), n (%)	505 (69)	232 (67)	39 (57)	0.100
Laboratory reports				
Triglycerides (mg/dL)	183.02 ± 180.98	191.65 ± 184.53	181.65 ± 94.46	0.745
Total cholesterol (mg/dL)	192.56 ± 43.77	192.55 ± 44.18	189.53 ± 50.84	0.861
HDL cholesterol (mg/dL)	43.64 ± 11.53	42.90 ± 11.96	42.32 ± 10.33	0.471
LDL cholesterol (mg/dL)	115.82 ± 36.53	115.93 ± 38.51	113.80 ± 39.61	0.911
HOMA-IR	7.08 ± 9.81	6.83 ± 9.26	7.33 ± 7.28	0.884
Glycosylated hemoglobin (%)	6.20 ± 1.20	6.07 ± 1.00	6.14 ± 1.09	0.202
Alanine aminotransferase (U/L)	74.27 ± 49.33	65.00 ± 43.05	49.19 ± 35.33	<0.001
Aspartate aminotransferase (U/L)	53.93 ± 33.64	48.38 ± 30.91	38.65 ± 16.86	<0.001
Histology scores				
Steatosis, n (%)				<0.01
<5%	19 (2.6)	2 (0.6)	0 (0)	
5–33%	281 (38.1)	117 (33.6)	22 (32.4)	
33–66%	269 (36.5)	120 (34.5)	24 (35.2)	
>66%	168 (22.8)	109 (31.3)	22 (32.4)	
Lobular inflammation, n (%)				<0.01
No foci	1 (0.1)	1 (0.3)	0 (0)	
<2 foci/200×	356 (48.3)	204 (58.6)	46 (67.6)	
2–4 foci/200×	295 (40)	110 (31.6)	14 (20.6)	
>4 foci/200×	85 (11.5)	33 (9.5)	8 (11.8)	
Ballooning, n (%)				0.037
None	231 (31.3)	124 (35.6)	28 (41.2)	
Few	204 (27.7)	100 (28.8)	16 (23.5)	
Many	302 (41)	124 (35.6)	24 (35.3)	
Fibrosis stages, n (%)				0.032
0	154 (20.9)	86 (24.7)	18 (26.5)	
1	193 (26.2)	102 (29.3)	18 (26.5)	
2	154 (20.9)	71 (20.5)	13 (19)	
3	155 (21)	61 (17.5)	11 (16.2)	
4	81 (11)	28 (8)	8 (11.8)	
Steatohepatitis, n (%)				<0.01
None	135 (18.3)	85 (24.4)	17 (25)	
Borderline steatohepatitis	144 (19.5)	67 (19.3)	16 (23.5)	
Definite steatohepatitis	458 (62.2)	196 (56.3)	35 (51.5)	
Steatohepatitis, n (%)[a]	602 (82)	263 (76)	51 (75)	0.018
Significant fibrosis (stage ≥2), n (%)	390 (53)	160 (46)	32 (47)	0.047

HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein; PNPLA3, patatin-like phospholipase domain-containing protein 3.
[a]It represents a composite of borderline and definite steatohepatitis.

Table 2. Multivariable association of *HSD17B13* rs72613567 or selected moderators with histology severity

	Steatosis (0–3)		Lobular inflammation (0–3)		Ballooning (0–2)	
	Odds ratio (95% CI)	*P* value[a]	Odds ratio (95% CI)	*P* value[a]	Odds ratio (95% CI)	*P* value[a]
Exposure						
HSD17B13 (additive model)						
rs72613567 (-/A) or (A/A)[b]	1.35 (1.13–1.61)	<0.01	0.69 (0.57–0.84)	<0.001	0.80 (0.67–0.97)	0.022
HSD17B13 (dominant model)						
rs72613567 (−/A) + (A/A)[b]	1.47 (1.17–1.84)	<0.01	0.65 (0.51–0.83)	<0.001	0.79 (0.63–0.99)	0.049
Potential moderators						
Age (yr)	0.97 (0.96–0.98)	<0.001	0.99 (0.98–1.00)	0.116	1.01 (1.00–1.02)	0.023
Body mass index (kg/m^2)	0.99 (0.98–1.01)	0.290	0.98 (0.97–1.00)	0.225	1.03 (1.02–1.05)	<0.001
Sex (women)	1.20 (0.95–1.52)	0.122	1.60 (1.25–2.06)	<0.001	1.54 (1.22–1.95)	<0.001
Type 2 diabetes (yes)	1.13 (0.89–1.43)	0.298	1.37 (1.06–1.75)	0.013	2.26 (1.77–2.89)	<0.001
PNPLA3 (dominant model)						
rs738409 (GC) + (GG)	1.43 (1.14–1.80)	0.002	1.34 (1.05–1.72)	0.018	0.95 (0.75–1.20)	0.686

	Steatohepatitis categories (0–2)		Overall fibrosis (0–4)		Significant fibrosis (stage ≥2)	
	Odds ratio (95% CI)	*P* value[a]	Odds ratio (95% CI)	*P* value[a]	Odds ratio (95% CI)	*P* value[c]
Exposure						
HSD17B13 (additive model)						
rs72613567 (−/A) or (A/A)[b]	0.77 (0.64–0.94)	0.010	0.84 (0.70–1.00)	0.050	0.82 (0.67–1.01)	0.068
HSD17B13 (dominant model)						
rs72613567 (−/A) + (A/A)[b]	0.75 (0.59–0.96)	0.022	0.80 (0.64–0.99)	0.046	0.78 (0.60–1.01)	0.061
Potential moderators						
Age (yr)	1.01 (1.00–1.02)	0.011	1.03 (1.02–1.04)	<0.001	1.04 (1.03–1.05)	<0.001
Body mass index (kg/m^2)	1.03 (1.01–1.05)	<0.01	1.03 (1.02–1.05)	<0.001	1.04 (1.02–1.06)	<0.001
Sex (women)	1.20 (0.93–1.54)	0.153	0.99 (0.79–1.24)	0.988	0.99 (0.75–1.30)	0.963
Type 2 diabetes (yes)	2.42 (1.84–3.19)	<0.001	2.55 (2.02–3.22)	<0.001	2.61 (1.98–3.44)	<0.001
PNPLA3 (dominant model)						
rs738409 (GC) + (GG)	0.95 (0.73–1.23)	0.708	1.36 (1.09–1.71)	<0.01	1.55 (1.18–2.03)	<0.01

The following variables were included in all multivariable analyses: *HSD17B13* rs72613567, nonheavy alcohol intake, age, sex, body mass index, T2D, and *PNPLA3* rs738409. CI, confidence interval.
[a]*P* values were calculated by ordered logistic regression.
[b]The reference genotype for comparison was (−/−).
[c]*P* values were calculated by binary logistic regression.

histology scores were similar to those found in the additive genetic model (Table 2).

Notably, carriage of the *HSD17B13* rs72613567 A-allele depicted opposite effects on fibrosis and steatosis severity (Table 1). Because steatosis and fibrosis scores were inversely correlated ($r = -0.10$, $P < 0.001$) in our data, we sought to investigate whether the protective effect of the rs72613567 A-allele on fibrosis was significantly mediated by a change in steatosis severity. Our causal mediation analysis confirmed that the rs72613567 A-allele might directly modify the risk of fibrosis severity (β coeff for total effect: −0.17, 95% CI: −0.31 to −0.02 and β coeff for direct effect: −0.17, 95% CI: −0.31 to −0.02 were similar) and not likely operate through steatosis (β coeff for indirect or steatosis-mediated effect: −0.0004, 95% CI: −0.02 to 0.02) (see Supplemental Table 1, Supplementary Digital Content 1, http://links.lww.com/CTG/A669).

HSD17B13 rs72613567 and risk of steatohepatitis and fibrosis at strata of *PNPLA3* rs738409

First, we sought to confirm the direction and strength of the association between *HSD17B13* rs72613567 (−/A) or (A/A) genotypes and histologic outcomes at strata of *PNPLA3* rs738409 genotypes (CC or GC + GG). A moderation analysis under an additive genetic model of *HSD17B13* rs72613567 showed that either *HSD17B13* rs72613567 (−/A) or (A/A) genotype was associated with a lower risk of steatohepatitis and fibrosis among individuals with *PNPLA3* rs738409-(CC) genotype. Nevertheless, this protection was not evident in patients with *PNPLA3* rs738409-(GC + GG) genotypes (see Supplemental Table 2 and Supplemental Figure 2A, Supplementary Digital Contents 1 and 2, http://links.lww.com/CTG/A669 and http://links.lww.com/CTG/A671). Further moderation analyses performed under a dominant genetic model of *HSD17B13* rs72613567 confirmed that the A-allele was

Table 3. Conditional effect of *HSD17B13* rs72613567 (−/A) + (A/A) genotypes on risk of steatohepatitis and fibrosis at levels of moderators

	Univariate analysis				Multivariable analysis[b]			
Moderators	Conditional effect[a]	Standard error	95% CI	*P* value	Conditional effect[a]	Standard error	95% CI	*P* value
Steatohepatitis categories (0–2)								
PNPLA3 rs738409								
CC, n = 377	−0.21	0.08	−0.37 to −0.04	0.014	−0.19	0.08	−0.35 to −0.03	0.019
GC + GG, n = 776	−0.09	0.06	−0.21 to 0.03	0.136	−0.08	0.05	−0.19 to 0.03	0.150
BMI ≥35, n = 506	−0.18	0.06	−0.29 to −0.06	<0.01	−0.17	0.06	−0.28 to −0.06	<0.01
BMI <35, n = 647	−0.03	0.09	−0.20 to 0.15	0.766	−0.003	0.09	−0.17 to 0.17	0.972
Age ≥45, n = 838	−0.18	0.06	−0.30 to −0.07	<0.01	−0.18	0.06	−0.29 to −0.07	<0.01
Age <45, n = 315	0.02	0.09	−0.16 to 0.20	0.845	0.02	0.09	−0.16 to 0.19	0.851
Women, n = 727	−0.18	0.06	−0.30 to −0.06	<0.01	−0.18	0.06	−0.29 to −0.06	<0.01
Men, n = 426	−0.04	0.08	−0.19 to 0.12	0.661	−0.03	0.08	−0.18 to −013	0.751
T2D (yes), n = 408	−0.18	0.08	−0.34 to −0.02	0.026	−0.18	0.08	−0.34 to −0.03	0.022
T2D (no), n = 745	−0.09	0.06	−0.21 to 0.03	0.130	−008	0.06	−0.20 to 0.03	0.145
Fibrosis (0–4)								
PNPLA3 rs738409								
CC, n = 377	−0.50	0.13	−0.77 to −0.23	<0.001	−0.45	0.12	−0.70 to −0.20	<0.001
GC + GG, n = 776	−0.03	0.09	−0.22 to 0.16	0.735	−0.008	0.09	−0.18 to 0.17	0.932
BMI ≥35, n = 506	−0.28	0.09	−0.46 to −0.09	<0.01	−0.22	0.08	−0.40 to −0.05	0.010
BMI <35, n = 647	−0.01	0.14	−0.30 to 0.27	0.921	0.002	0.13	−0.26 to 0.27	0.991
Age ≥45, n = 838	−0.24	0.09	−0.42 to −0.06	0.010	−0.23	0.09	−0.40 to −0.06	<0.01
Age <45, n = 315	−0.05	0.14	−0.34 to 0.23	0.711	−0.04	0.14	−0.31 to 0.24	0.799
Women, n = 727	−0.19	0.09	−0.39 to 0.00	0.051	−0.17	0.09	−0.35 to 0.01	0.067
Men, n = 426	−0.18	0.12	−0.43 to 0.07	0.162	−0.14	0.12	−0.37 to 0.10	0.262
T2D (yes), n = 408	−0.33	0.12	−0.58 to −0.08	0.010	−0.32	0.12	−0.56 to −0.07	0.011
T2D (no), n = 745	−0.10	0.09	−0.28 to 0.09	0.291	−0.07	0.09	−0.2 to 0.11	0.429

The reference genotype for all comparative analyses *HSD17B13* rs72613567 (−/−).
BMI, body mass index; CI, confidence interval; T2D, type 2 diabetes.
[a]Represents the effect of the exposure (*HSD17B13* rs72613567 (−/A) + (A/A) genotypes) on outcomes at values of the moderator (i.e., *PNPLA3* rs738409 genotypes (CC) or (GC) + (GG)).
[b]Analysis adjusted for nonheavy alcohol intake and those covariates not tested as moderators.

associated with remarkable protective effects on risk of steatohepatitis (β coeff: −0.19, $P = 0.019$) and fibrosis (β coeff: −0.45, $P < 0.001$) among individuals with *PNPLA3* rs738409-(CC) genotype; however, this protection was fully mitigated among those with *PNPLA3* rs738409-(GC) + (GG) genotypes (Table 3).

Figure 1a shows the difference in the risk of significant fibrosis between individuals with *HSD17B13* rs72615367 (−/A) + (A/A) and (−/−) genotypes at strata of *PNPLA3* rs738409 genotypes. In the presence of *PNPLA3* rs738409-(CC) genotype, the risk of significant fibrosis was significantly reduced among carriers of *HSD17B13* rs72613567 A-allele as compared with homozygous for the A-allele deletion (−/−) (adjusted risk difference −15, 95% CI: −24 to −6; $P < 0.001$). By contrast, the risk of significant fibrosis was not significantly different between *HSD17B13* rs72613567 A-allele carriers and homozygous (−/−) in patients with *PNPLA3* rs738409-(CC) + (GG) genotypes (adjusted risk difference −0.7, 95% CI: −7 to 6; $P = 0.821$).

Figure 2 depicts the association between *HSD17B13* rs72613567 and risk of steatohepatitis (Panel A) or fibrosis (Panel B) at strata of moderators while controlling for relevant confounders. The *HSD17B13* rs72613567 A-allele lowers the risk of steatohepatitis (adjusted OR: 0.61, $P = 0.024$) and fibrosis (adjusted OR: 0.48, $P < 0.001$) in those with *PNPLA3* rs738409-(CC) genotype. However, the protective effect of *HSD17B13* rs72613567 A-allele on risk of steatohepatitis (adjusted OR: 0.82, $P = 0.208$) and fibrosis (adjusted OR: 1.00, $P = 0.979$) was no longer evident among individuals with *PNPLA3* rs738409-(GC) + (GG) genotypes.

HSD17B13 rs72613567 and risk of steatohepatitis and fibrosis at strata of traditional risk factors

Moderation effects of BMI. We first sought to illustrate graphically the influence of a 2-way interaction term of *HSD17B13* rs72613567 (−/A) + (A/A) or (−/−) with BMI on the risk of steatohepatitis while adjusting for relevant confounders (Figure 1b). As shown in

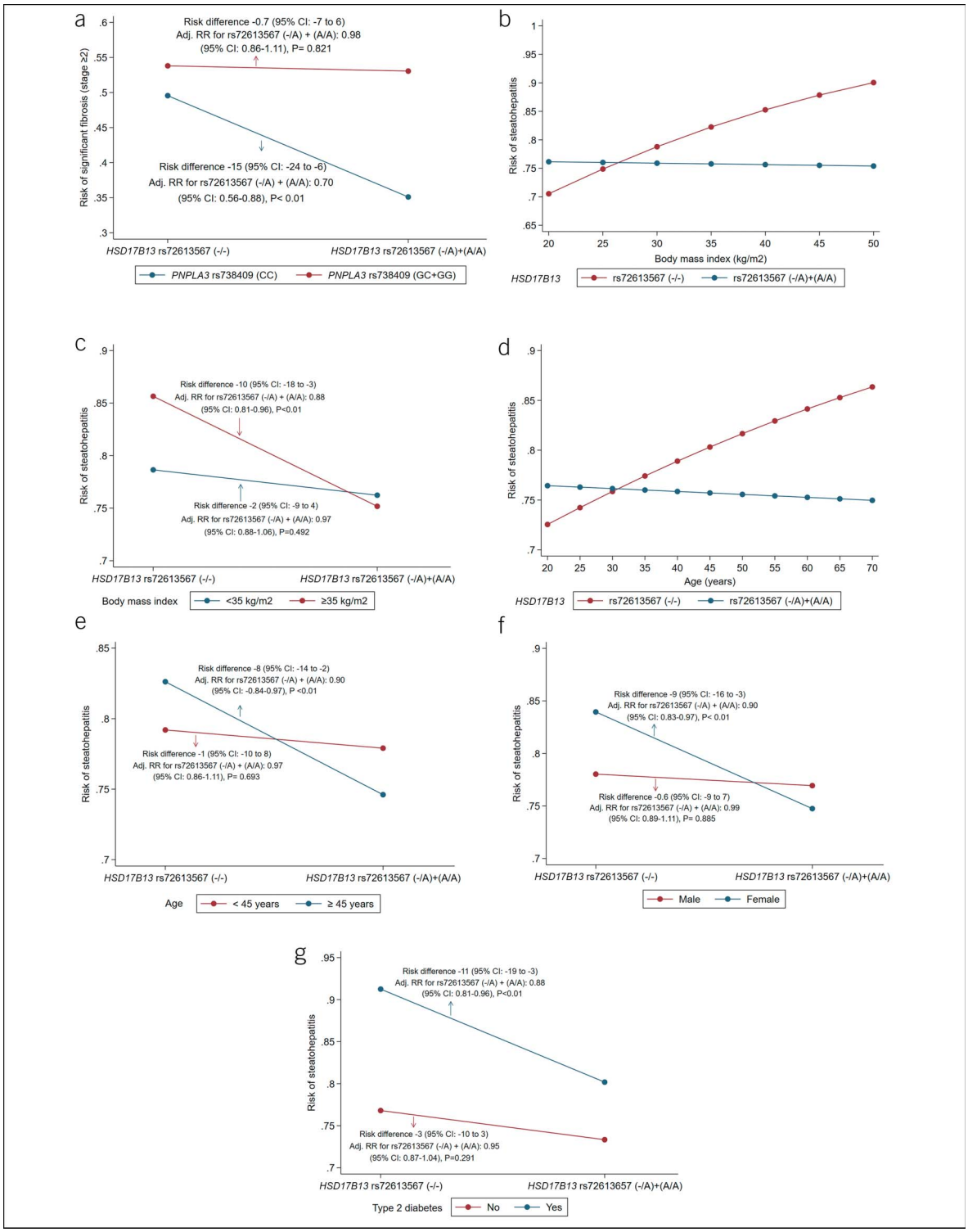

Figure 1. Effect of *HSD17B13* rs72613567 (dominant genetic model) on risk of significant fibrosis or steatohepatitis at levels of moderators. Analysis based on margins plots. (**a**) *PNPLA3* rs738409 (CC, n = 377, and GC + GG, n = 776). (**b**) Body mass index (continuous). (**c**) Body mass index (<35, n = 647, and ≥ 35, n = 506). (**d**) Age (continuous). (**e**) Age (<45, n = 315, and ≥ 45, n = 838). (**f**) Sex (women, n = 727, and men, n = 426). (**g**) Type 2 diabetes (no, n = 745, and yes, n = 408). CI, confidence interval; RR, relative risk. The risk of steatohepatitis includes borderline along with definite steatohepatitis. Analysis adjusted for nonheavy alcohol intake and those covariates not tested as moderators. The reference for all comparative analyses is the *HSD17B13* rs72613567 (−/−) genotype. Logistic regression models were used to compute risks of steatohepatitis or significant fibrosis, and margins command to create a visual display of results. Circles on the lines represent average adjusted risks of steatohepatitis or significant fibrosis according to *HSD17B13* rs72613567 (−/−) and (−/A) + (A/A) genotypes. Risk difference indicates a change in the risk of having steatohepatitis or significant fibrosis for *HSD17B13* rs72613567 (−/A) + (A/A) as compared with (−/−) at levels of moderators.

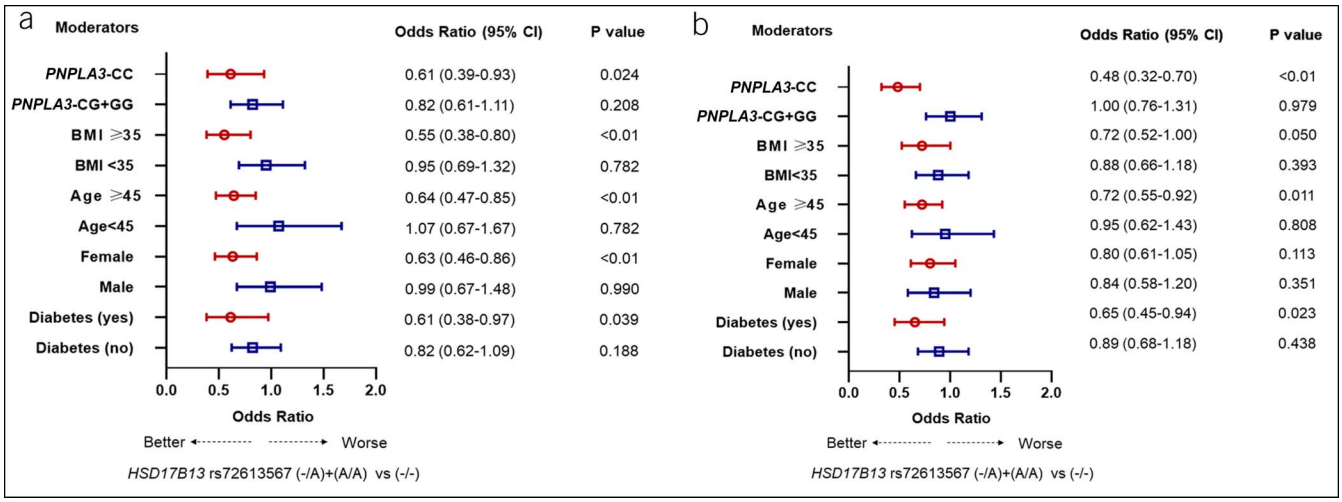

Figure 2. Effect of *HSD17B13* rs72613567 (dominant genetic model) on risk of steatohepatitis and fibrosis. Adjusted risk at strata of moderators' cutoffs. Results based on ordered logistic regression. (**a**) Risk of steatohepatitis (0–2). (**b**) Risk of fibrosis (0–4). BMI, body mass index, NASH, nonalcoholic steatohepatitis. Analysis adjusted for nonheavy alcohol intake and those covariates not tested as moderators. The reference for all comparative analyses was the *HSD17B13* rs72613567 (−/−) genotype.

this figure, higher BMI was associated with a greater risk of steatohepatitis among individuals with (−/−) genotype (Adj OR: 1.03, $P = 0.017$); however, BMI did not seem to impact significantly on risk of steatohepatitis in carriers of *HSD17B13* rs72613567 A-allele (adjusted OR: 1.01, $P = 0.144$). Of note, the risk of steatohepatitis between (−/−) and (-/A) + (A/A) genotypes were significantly different in those who had a BMI of ≥35 ($P < 0.05$ for risk differences at BMI values of 35, 40, 45, and ≥50).

Under an additive genetic model of *HSD17B13* rs72613567, both (−/A) and (A/A) genotypes were significantly linked to a lower risk of steatohepatitis in patients with BMI ≥35 (see Supplemental Table 3 and Supplemental Figure 2B, Supplementary Digital Contents 1 and 2, http://links.lww.com/CTG/A669 and http://links.lww.com/CTG/A672). However, the protection of *HSD17B13* rs72613567 A-allele was no longer significant in patients with BMI <35. We replicated our moderation analysis using a dominant genetic model of *HSD17B13* rs72613567 (Table 3). This analysis confirmed strong protection of the A-allele on steatohepatitis and fibrosis only in those with a BMI of ≥ 35. When compared with *HSD17B13* rs72613567 (−/−) genotype, the presence of the A-allele was associated with a reduced risk of steatohepatitis only in patients with BMI ≥35 (adjusted risk difference: −10, 95% CI: −18 to −3; $P < 0.01$) (Figure 1c). Furthermore, the protective effect of A-allele remained significantly associated with lower risk of steatohepatitis (adjusted OR: 0.55, $P < 0.01$) and fibrosis (adjusted OR: 0.72, $P = 0.050$) but only in patients with BMI ≥ 35 (Figures 2a,b).

Table 4. Conditional effect of *HSD17B13* rs72613567 (−/A) + (A/A) genotypes on risk of steatohepatitis and fibrosis at levels of 2 moderators (sex and age <51 or ≥ 51 yr)

	Univariate analysis				Multivariable analysis[b]			
Moderators	Conditional effect[a]	Standard error	95% CI	*P* value	Conditional effect[a]	Standard error	95% CI	*P* value
Steatohepatitis categories (0–2)								
Men/<51, n = 238	0.05	0.11	−0.16 to 0.26	0.619	0.06	0.10	−0.15 to 0.26	0.584
Men/≥51, n = 188	−0.15	0.12	−0.39 to 0.08	0.190	−0.11	0.11	−0.34 to 0.11	0.327
Women/<51, n = 286	−0.03	0.09	−0.21 to 0.16	0.756	−0.02	0.09	−0.20 to 0.16	0.834
Women/≥51, n = 441	−0.28	0.08	−0.44 to −0.12	0.001	−0.31	0.08	−0.46 to −0.15	<0.001
Fibrosis (0–4)								
Men/<51, n = 238	−0.09	0.17	−0.43 to 0.24	0.567	−0.08	0.16	−0.40 to 0.24	0.624
Men/≥51, n = 188	−0.31	0.19	−0.68 to 0.06	0.103	−0.22	0.18	−0.57 to 0.14	0.232
Women/<51, n = 286	−0.15	0.15	−0.45 to 0.14	0.317	−0.11	0.14	−0.39 to 0.18	0.459
Women/≥51, n = 441	−0.17	0.13	−0.42 to 0.08	0.196	−0.23	0.12	−0.47 to 0.01	0.062

The reference genotype for all comparative analyses *HSD17B13* rs72613567 (−/−). CI, confidence interval.
[a]Represents the effect of the exposure (*HSD17B13* rs72613567 (−/A) + (A/A) genotypes) on outcomes at values of moderators.
[b]Analysis adjusted for *PNPLA3* rs738409, nonheavy alcohol intake, body mass index, and type 2 diabetes.

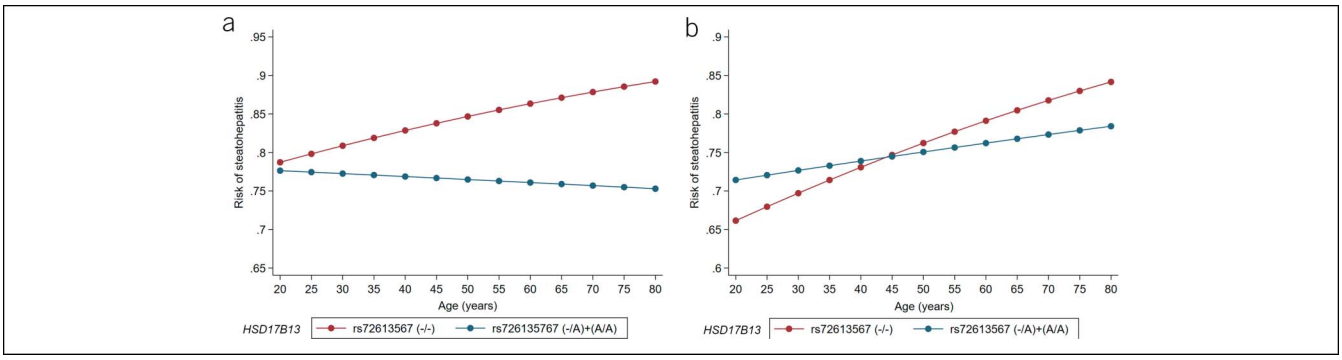

Figure 3. Effect of *HSD17B13* rs72613567 (dominant genetic model) on the risk of steatohepatitis at different cutoffs of age (years). Sensitivity analysis based on sex: (**a**) women*, (**b**) men†. *Risk of steatohepatitis started to be significantly different ($P < 0.05$) between (−/−) and (−/A) + (A/A) at age ≥50 years. †Risk of steatohepatitis was not significantly different ($P > 0.05$) between (−/−) and (−/A) + (A/A) across all age cutoffs.

Moderation effects of age. As we did with the analysis of BMI, the effect of a two-way interaction term of *HSD17B13* rs72613567 (−/A) + (A/A) and (−/−) with age on the risk of steatohepatitis was explored through margins analysis. Figure 1d displays a positive and significant association between age and risk of steatohepatitis among those with *HSD17B13* rs72613567 (−/−) genotype (adjusted OR: 1.01, $P = 0.050$). However, the effect of age on the risk of steatohepatitis was significantly attenuated among individuals carrying the A-allele (adjusted OR: 1.005, $P = 0.460$). It is important to note that the risk of steatohepatitis between (−/−) and (−/A) + (A/A) genotypes started to be significantly different at an age cutoff of 45 years or older (all P values < 0.05). Thus, further sensitivity analyses were performed to better understand the effect of *HSD17B13* rs72613567 on histological outcomes between patients aged younger than 45 years and 45 years or older.

Supplemental Table 4 and Supplemental Figure 2C, Supplementary Digital Contents 1 and 2, http://links.lww.com/CTG/A669 and http://links.lww.com/CTG/A673, show that protective effects of both rs72613567 (−/A) and (A/A) genotypes on the risk of steatohepatitis were only evident among those aged 45 years or older. Further moderation analyses under a dominant genetic model of *HSD17B13* rs72613567 confirmed strong and significant protective effects of the A-allele on risk of steatohepatitis and fibrosis among those aged 45 years or older (Table 3).

Among patients aged 45 years or older, the risk of steatohepatitis was significantly lower (adjusted risk difference: −8, 95% CI: −14 to −2; $P < 0.01$) in carriers of the *HSD17B13* rs72613567 A-allele than in those with (−/−) genotype. Among people younger than 45 years, the risk of steatohepatitis did not significantly differ between (−/−) and (−/A) + (A/A) genotypes (adjusted risk difference: −1, 95% CI: −10 to 8; $P = 0.693$) (Figure 1e). A further sensitivity analysis looking at the association between *HSD17B13* rs72613567 and histologic outcomes at strata of age (<45 or ≥45) showed strong protection of the A-allele against risk of steatohepatitis (adjusted OR: 0.64, $P < 0.01$) and fibrosis (adjusted OR: 0.72, $P = 0.011$) only in those aged 45 years or older (Figures 2a,b).

Moderation effects of sex. To confirm whether sex might moderate the effect of *HSD17B13* rs72613567 on histologic outcomes, we first tested the nature of this relationship under an additive genetic model (see Supplemental Table 5 and Supplemental Figure 2D, Supplementary Digital Contents 1 and 2, http://links.lww.com/CTG/A669 and http://links.lww.com/CTG/A674). Among women, those with *HSD17B13* rs72613567 (−/A) or (A/A) genotypes had a significantly lower risk of steatohepatitis and fibrosis when compared with those with (−/−) genotype. Nonetheless, this protective effect was not significant among men. The same analysis performed under a dominant genetic model of *HSD17B13* rs72613567 confirmed a notable protective effect of the A-allele on risk of steatohepatitis exclusively among women (Table 3). For example, the effect of *HSD17B13* rs72613567 A-allele on risk of steatohepatitis was estimated to be −0.18 ($P < 0.01$) and −0.03 ($P = 0.751$) in women and men, respectively.

Figure 1f clearly shows that women with the A-allele had a reduced risk of steatohepatitis (adjusted risk difference: −9, 95% CI: −16 to −3; $P < 0.01$) compared with those with (−/−) genotype. Among men, the risk of steatohepatitis was not significantly different between (−/−) and (−/A) + (A/A) genotypes (adjusted risk difference: −0.6, 95% CI: −9 to 7; $P = 0.885$). Covariate-adjusted logistic regression analyses validated our previous analyses and confirmed that protection conferred by the A-allele on the risk of steatohepatitis (adjusted OR: 63, $P < 0.01$) was particularly stronger in women (Figure 2a).

Because a decline in estrogen has been implicated with NAFLD progression among postmenopausal women and given the potential association of 17β-HSD13 with sex hormone metabolism, we sought to explore whether the effect of *HSD17B13* rs72613567 on the risk of steatohepatitis and fibrosis might differ between women aged younger than 51 or 51 years or older; the median age for reaching menopause among non-Hispanic white women in the United States is 51 years (37). Our moderation analyses based on a dominant genetic model of *HSD17B13* rs72613567 showed stronger protective effects of the A-allele against risk of steatohepatitis in women aged 51 years or older (β coeff: −0.31, $P < 0.001$) vs younger than 51 years (β coeff: −0.02, $P = 0.834$). The protection of the A-allele on risk of steatohepatitis and fibrosis did not significantly differ among men aged younger than 51 years or 51 years or older (Table 4). Figure 3 shows the effect of *HSD17B13* rs72613567 on the risk of steatohepatitis at different strata of age and sex. Among women, the risk of steatohepatitis started to be significantly different ($P < 0.05$) between (−/−) and (−/A) + (A/A) genotypes at age 50 years or older (Figure 3a). However, the risk of steatohepatitis did not differ between rs72613567 (−/−) and (-/A) + (A/A) genotypes across all age cutoffs among men (Figure 3b).

Moderation effects of type 2 diabetes mellitus. Compared with *HSD17B13* rs72613567 (−/−) genotype, risks of steatohepatitis

in patients with (−/A) or (A/A) genotypes were significantly lower among patients with diabetes than without diabetes (see Supplemental Table 6 and Supplemental Figure 2E, Supplementary Digital Contents 1 and 2, http://links.lww.com/CTG/A669 and http://links.lww.com/CTG/A675). Under a dominant genetic model of *HSD17B13* rs72613567, our moderation analyses confirmed that only individuals with diabetes and carrying the A-allele might be protected against steatohepatitis (β coeff: −0.18; $P = 0.022$) and fibrosis (β coeff: −0.32; $P = 0.011$). However, the protection of the A-allele was no longer evident among those without diabetes (Table 3).

Two further sensitivity analyses confirmed that protective effects of *HSD17B13* rs72613567 A-allele on the risk of steatohepatitis and fibrosis were seen only in participants with diabetes. Among individuals with diabetes, the carriage of rs72613567 A-allele was associated with a reduced risk of steatohepatitis when compared with those with (−/−) genotype (adjusted risk difference: −11, 95% CI: −19 to −3; $P < 0.01$). However, risk of steatohepatitis was not significantly different between (−/A) + (A/A) and (−/−) genotypes (adjusted risk difference: −3, 95% CI: −10 to 3; $P = 0.291$) in those without diabetes (Figure 1g). The presence of the A-allele was only associated with lower risk of steatohepatitis (adjusted OR: 0.61, $P = 0.039$) and fibrosis (adjusted OR: 0.65, $P = 0.023$) among individuals with diabetes (Figures 2a,b).

The association between *HSD17B13* rs72613567 and histologic scores remained significant in the overall population and those selected strata of traditional risk factors after adjusting for multiple testing (see Supplemental Tables 7 and 8, Supplementary Digital Content 1, http://links.lww.com/CTG/A669).

In a separate analysis, we explored the relationship between *HSD17B13* rs72613567 and liver histology severity in 156 Hispanics, 106 non-Hispanic Asians, and 52 non-Hispanic blacks with NAFLD whose liver histology was centrally reviewed by the NASH CRN pathology subcommittee. We observed that *HSD17B13* rs72613567 was positively associated with steatosis ($P = 0.02$) and inversely linked with fibrosis stages ≥ 2 ($P = 0.049$) in Hispanics but not in the other 2 racial groups (data not shown).

DISCUSSION

Two common polymorphisms in *HSD17B13* (rs72613567) and *PNPLA3* (rs738409) have been strongly implicated in the risk of progression to steatohepatitis and fibrosis, including end-stage of liver disease in patients with NAFLD and alcoholic liver disease (2–4,6–9). Both variants, *HSD17B13* rs72613567 and *PNPLA3* rs738409, have opposite effects on the risk of NAFLD progression. However, whether *HSD17B13* rs72613567 or *PNPLA3* rs738409 may neutralize or attenuate the effect of each other on the risk of steatohepatitis and fibrosis is unclear. Beyond this gene–gene interaction, how the relationship between *HSD17B13* rs72613567 and other major determinants of the natural course of NAFLD including age, sex, BMI, and diabetes might impact NAFLD progression has not been clarified either. Thus, this study aimed to elucidate how *PNPLA3* rs738409 and other clinical and demographic factors might modify the relationship between *HSD17B13* rs72613567 and risk of steatohepatitis and fibrosis.

As shown in previous studies, our data confirmed that the *HSD17B13* rs72613567 A-insertion allele protected against steatohepatitis and fibrosis in the analysis performed under additive and dominant genetic models (2,3,7). Although individuals carrying the *HSD17B13* rs72613567 A-allele had lesser risk of steatohepatitis and fibrosis, this protective effect was significantly different between individuals with *PNPLA3* rs738409 genotypes (GC + GG) and (CC). Of note, the beneficial effects of *HSD17B13* rs72613567 A-allele on steatohepatitis and fibrosis were fully mitigated in patients carrying the *PNPLA3* rs738409 G-allele. Further analyses showed that associations between the *HSD17B13* rs72613567 A-allele and histology scores were also significantly different depending on age, BMI, sex, and diabetes status. The protective effects of *HSD17B13* rs72613567 A-allele on risk of inflammation and fibrosis were particularly stronger in women, persons aged 45 years or older, individuals with diabetes, or those with BMI ≥ 35, even after adjusting for the other relevant confounders.

Noteworthy, the severity of steatosis was significantly higher in carriers of the *HSD17B13* rs72613567 A-allele compared with noncarriers, a finding previously described (3,7). Because severity in fibrosis and steatosis were inversely correlated in our cohort and one can expect that advanced liver fibrosis is often accompanied by a reduction in hepatic fat to the point of complete fat loss (38), we sought to investigate whether the effect of *HSD17B13* rs72613567 on fibrosis severity was mediated by a change in steatosis severity. Our causal mediation analysis showed a direct effect of the *HSD17B13* rs72613567 A-allele on fibrosis severity. In other words, the protective effect of this *HSD17B13* variation on fibrosis was not significantly mediated by a change in steatosis. In support of our findings, Ma et al. have suggested that 17β-HSD13 does not directly regulate hepatocyte lipid content (7).

The effect of *PNPLA3* rs738409 and *HSD17B13* rs72613567 interactions on ALT and AST levels has been previously explored in 2 large studies including patients with biopsy-proven NAFLD along with other etiologies of chronic liver disease. Abul-Husn et al., using a large cohort of European descent, identified strong interactions between *HSD17B13* rs72613567 and *PNPLA3* rs738409 in association with ALT and AST levels (3). They reported that the presence of *HSD17B13* rs72613567 A-allele mitigated increases in ALT and AST levels among carriers of *PNPLA3* rs738409 G-allele. RNA sequencing–based expression analysis revealed that *HSD17B13* rs72613567 was associated with decreased *PNPLA3* messenger RNA expression in an allele dose-dependent manner. However, this study did not report interaction effects between these 2 variants and their association with liver histology severity. Another study recently published, which included white patients with biopsy-confirmed NAFLD from the NASH-CRN observational database and PIVENS study and those seen at the NIH Clinical Center, did not find a significant interaction between *PNPLA3* rs738409 and *HSD17B13* variants, although authors stated that the study was not powered to detect such an interaction effect (7). Interestingly, in this study, as illustrated through their supplemental figure 6, they noticed that the effect of *HSD17B13* rs72613567 A-allele on ballooning, lobular inflammation, and fibrosis was attenuated in patients with the *PNPLA3* rs738409 G-allele (Figure 6 of Supplementary Material, Supplementary Digital Content 1, http://links.lww.com/CTG/A669 in the study by Ma et al. manuscript) (7).

Although this analysis included the same NAFLD population (NASH CRN) as in the study by Ma et al. (7), we included a larger proportion of non-Hispanic whites (n = 1,153), which may significantly increase the statistical power to detect statistically significant interactions or moderation effects between *HSD17B13* rs72613567, *PNPLA3* rs738409, and other phenotypic risk factors.

17β-HSD13 has been found to associate with LDs and is significantly upregulated in the liver of patients with NAFLD

(2,3,7,12). Most recently, 17β-HSD13 has been reported to regulate retinoid metabolism by acting as a retinol dehydrogenase (7,39). Most of the retinol present in the body is stored as retinyl esters within LD in HSCs, and it may play a critical role in the activation of HSCs, which may lead to fibrosis (40). *HSD17B13* rs72613567 is supposed to alter mRNA splicing that results in a truncated protein with absent or reduced enzymatic activity. This loss-of-function mutation in the *HSD17B13* gene confers a strong protective effect on inflammation, fibrosis, cirrhosis, and even hepatocellular carcinoma (2,3,5–9,41).

On the other hand, *PNPLA3* rs738409 has been associated with an increased risk of NAFLD and its severity (1,15). The *PNPLA3* rs738409 has been linked to impaired hydrolysis of triglycerides and LD remodeling in hepatocytes, which may lead to hepatic steatosis (1). Besides, PNPLA3 might also participate in the metabolism of retinoids. It seems to act as a lipase responsible for retinyl-palmitate hydrolysis in HSCs in humans. In the presence of *PNPLA3* rs738409 variation, there is reduced activity of retinyl palmitate lipase enzyme, resulting in liver fibrosis (17).

Interestingly, both PNPLA3 and 17β-HSDB13 are involved in LD remodeling and retinoid acid (RA) metabolism pathways in the liver, which might suggest biologically plausible ways for this gene–gene interaction, although either genetic variant may impact the functionality of liver-specific LD-associated proteins or RA metabolism in opposite directions. A better understanding of the pathogenic role of this gene–gene interaction needs to be addressed in further studies.

The role of 17β-HSD13 in the regulation of sex steroid metabolism is not fully understood in humans. Adam et al. (14) recently reported that knockout of *HSD17B13* in mice does not affect reproductive performance and sex hormone concentrations. Unlike human studies, they noted that hepatic steatosis and inflammation were significantly increased in mice with reduced *HSD17B13* expression (14). It is noteworthy that the nonprotective effect of *HSD17B13* knockout was only studied in male mice. Thus, whether the knockout of *HSD17B13* may induce similar or different histologic phenotypes in female and male mice remains to be elucidated.

In this present study, we observed that the protection conferred by the *HSD17B13* rs72613567 A-allele on risk of steatohepatitis was only evident in women aged 51 years or older compared with those aged younger than 51 years, even after adjusting for relevant clinical confounders such as BMI and T2D. The exact mechanism by which *HSD17B13* rs72613567 might protect against NAFLD severity women older than 51 years remains to be elucidated. Low serum concentrations of RA and/or carotenoids have been documented in obesity (42), metabolic syndrome (43), and T2D (44). Low levels of RA and carotenoids have also been implicated in the pathogenesis of NAFLD (45–47). RA seems to be an important regulator of HSCs activation, which is a major contributor to liver fibrosis (40). Recently, 17β-HSD13 has been reported to regulate retinoid metabolism by acting as a retinol dehydrogenase (7). Thus, there is a potential biologic link to explain partly the protective effect of *HSD17B13* rs72613567 on inflammation and fibrosis in NAFLD. Patients with obesity and those with metabolic syndrome tend to have a higher risk of NAFLD severity (48). In women, menopause is associated with estrogen depletion, which has been linked to stellate cell activation and fibrogenesis (49). In addition, postmenopausal women are more likely to develop visceral obesity, insulin resistance, and other features of metabolic syndrome, including dyslipidemia, high blood pressure, and T2D; all of them are strongly associated with increased risk of NAFLD and its progression (50). The abovementioned associations between obesity, sex (particularly postmenopausal women), metabolic syndrome, RA and/or carotenoids levels, stellate cell function, and NAFLD severity may mechanistically support the hypothesis that *HSD17B13* 72613567 may confer its higher protection to patients at increased risk of NAFLD progression by likely impacting the retinol metabolism and LD remodeling, although further studies are necessary to establish whether *HSD17B13* rs72613567 might also exert its protection by affecting sex steroid metabolism.

To our knowledge, this is the first attempt to provide novel insight into how the protective effects of *HSD17B13* rs72613567 against steatohepatitis and fibrosis severity may vary across a wide subgroup of patients at higher risk of NAFLD progression. These findings are particularly important regarding risk predictions because patients aged 45 years and older, those with obesity or T2D and postmenopausal women have been consistently associated with an accelerated clinical course of the disease with worse clinical outcomes (48,50–52). This also supports the idea that the explanatory power of genetic variants could be enhanced by combining them with other sources of individual variation, such as demographic risk factors or comorbidities, as shown in this study. Thus, our findings may have notable implications: from a public health perspective, identifying the subset of individuals in whom a preventive or therapeutic intervention could have the most beneficial effect; and regarding risk estimates, improving the fit of the model or its predictive capability by the inclusion of interaction terms in multivariate models.

Gene–gene or gene–phenotype interactions add complexity to the already complex individual roles of gene and environmental factors in the pathogenesis of NAFLD. Unfortunately, our study was not initially designed to explore the molecular mechanisms to explain these complex interactions. Thus, further experimental studies may help to unravel the mechanisms whereby these complex interactions may occur. Our results are based on non-Hispanic whites; hence, replication of our findings in independent multiethnic cohorts would be required. Although the effect sizes of our associations seemed to be relatively large, stratified analysis performed in our analyses might reduce the power of the study, resulting in a nondifferential bias underestimating the real size effect. To minimize this limitation, all moderation analyses used the methodology recommended by Preacher and Hayes (32), which uses bootstrapping, a nonparametric resampling procedure, because it is considered the most powerful, most effective method to use with small samples, and the least vulnerable to type I error. Furthermore, associations between *HSD17B13* rs72613567 and histologic scores remained significant in the overall population and those selected strata of traditional risk factors after performing multiple comparisons adjustments.

In conclusion, our study examined how the relationship between *HSD17B13* rs72613567 and NAFLD severity is modified by genetic, demographic, and clinical factors. Although *HSD17B13* rs72613567 remained significantly associated with lower risk of steatohepatitis and fibrosis in the whole cohort, this protective effect seemed to be significantly modified at different levels of other traditional risk factors with recognized association with NAFLD progression. The protection of the *HSD17B13* rs72613567 A-allele was significantly mitigated in carriers of the *PNPLA3* rs738409 G-allele. The major benefits of the *HSD17B13*

Study Highlights

WHAT IS KNOWN

- ✓ *HSD17B13* rs72613567 A-insertion allele may confer protection against nonalcoholic steatohepatitis and its progression.
- ✓ *PNPLA3* rs738409, age, body mass index, sex, and type 2 diabetes mellitus are strongly associated with the risk of nonalcoholic fatty liver disease progression.
- ✓ The effect of interactions between *HSD17B13* rs72613567, *PNPLA3* rs738409, and other phenotypic risk factors on risk of steatohepatitis and fibrosis is not well understood.

WHAT IS NEW HERE

- ✓ The presence of *PNPLA3* rs738409 G-allele mitigates the protective effect of *HSD17B13* rs7261537 A-allele on risk of steatohepatitis and fibrosis.
- ✓ The *HSD17B13* rs72613567 A-allele might confer stronger protection against steatohepatitis and fibrosis among individuals who are aged ≥ 45 years, women, and have class ≥ 2 obesity or diabetes.
- ✓ Among women, the protection of the *HSD17B13* rs72613567 A-allele is stronger in those aged 51 years and older.

rs72613567 A-allele were seen in patients at increased risk of NAFLD progression, including women (particularly those aged 51 years or older), individuals with T2D and those with BMI ≥35 or aged 45 years or older. Our data support the idea that identification of gene–gene or gene–phenotype moderation effects should be at the forefront in attempts to understand the pathophysiology of NAFLD, construct more accurate risk prediction models, and identify subsets of the population that would obtain the greatest benefit from therapeutic interventions.

CONFLICTS OF INTEREST

Guarantor of the article: Naga Chalasani, MD, had full access to all data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Specific author contributions: All authors made substantial contributions to the intellectual content of the paper and approved the final version of the manuscript. Conception and design: N.C. and E.V.-G. Acquisition of data: L.A.W., N.C., E.V.-G., and T.L. Analysis and interpretation of data: N.C., E.V.-G., S.S., C.J.P., and L.A.W. Drafting of the manuscript: N.C., E.V.-G., S.S., and C.J.P. Critical revision of the manuscript for intellectual content: N.C, E.V.-G., S.S., C.J.P., T.L., and L.A.W. Statistical analysis: E.V.-G., C.J.P. and S.S. Obtaining funding and supervision: N.C.

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Potential competing interests: There are none for this paper. For full disclosure, N.C. has ongoing paid consulting activities (or had in the preceding 12 months) with NuSirt, Abbvie, Afimmune (DS Biopharma), Allergan (Tobira), Madrigal, Coherus, Siemens, La Jolla, Foresite labs, and Genentech. These consulting activities are generally in the areas of nonalcoholic fatty liver disease and drug hepatotoxicity. N.C. receives research grant support from Exact Sciences, Intercept, and Galectin Therapeutics, where his institution receives the funding. Over the past decade, N.C. has served as a paid consultant to more than 35 pharmaceutical companies, and these outside activities have regularly been disclosed to his institutional authorities. All other authors have no reported conflicts.

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