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High Risk of Fatty Liver Disease Amplifies the Alanine Transaminase–Lowering Effect of a *HSD17B13* Variant

Helene Gellert-Kristensen,[1] Børge Grønne Nordestgaard,[2-5] Anne Tybjærg-Hansen,[1,3-5] and Stefan Stender [1]

A common loss-of-function variant in *HSD17B13* (rs72613567:TA) was recently found to protect from chronic liver disease. Whether the variant confers protection from specific risk factors for liver disease is unclear. We tested the association of rs72613567 with plasma levels of alanine transaminase (ALT) and clinical liver disease and mortality in 111,612 individuals from the Danish general population, including 497 with cirrhosis and 113 with hepatocellular carcinoma. *HSD17B13* rs72613567:TA was associated with stepwise lower levels of plasma ALT of up to 1.3 U/L in TA/TA homozygotes versus T/T homozygotes. For each TA-allele, the risk of cirrhosis and hepatocellular carcinoma was reduced by 15% and 28%, respectively. In prospective analyses, the TA-allele was associated with up to 33% lower rates of liver-related mortality in the general population, and with up to 49% reduced liver-related mortality in patients with cirrhosis. The ALT-lowering effect of rs72613567:TA was amplified by increasing adiposity, alcohol consumption, and genetic risk of fatty liver disease. The TA-allele was associated with only marginally lower ALT in lean nondrinkers with low genetic risk of hepatic steatosis. In contrast, compared with T/T homozygotes, TA/TA homozygotes had 12% to 18% lower plasma ALT among the most obese, in heavy drinkers, and in individuals carrying three or four steatogenic alleles in patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) and transmembrane 6 superfamily 2 (*TM6SF2*). *Conclusion:* High risk of fatty liver disease amplifies the ALT-lowering effect of *HSD17B13* rs72613567:TA in the Danish general population. (Hepatology 2019;0:1-11).

With a prevalence between 19% and 46%, fatty liver disease has reached epidemic proportions.[1-3] Hepatic steatosis, the first stage of the disease, is an asymptomatic and reversible condition characterized by an excess of triglycerides in hepatocytes. Prolonged exposure to hepatic steatosis increases the risk of liver inflammation (steatohepatitis), fibrosis, cirrhosis, and ultimately hepatocellular carcinoma, a cancer that kills more than 80% of those afflicted within 3 years of diagnosis.[4] Fatty liver disease is expected to become the most common indication for liver transplantation in the United States by 2020.[5]

Obesity and alcohol are the major risk factors for fatty liver disease, and the increasing prevalence of the disorder mirrors the rising rates of obesity worldwide.[6,7] Genetic factors likewise play an important role in fatty liver disease. Several genetic variants that promote the full spectrum of fatty liver disease have been identified in genome-wide association studies during the past decade. The most important of these are p.I148M in patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) and p.E167K in transmembrane 6 superfamily 2 (*TM6SF2*), two common missense variants that each increase the risk of hepatic steatosis, steatohepatitis, fibrosis, and cirrhosis by 2-fold to 4-fold.[8-11]

Abbreviations: ALT, alanine transaminase; BMI, body mass index; CCHS, Copenhagen City Heart Study; CGPS, Copenhagen General Population Study; CI, confidence interval; HSD17B13, hydroxysteroid 17-beta dehydrogenase 13; ICD, International Classification of Diseases; OR, odds ratio; PNPLA3, patatin-like phospholipase domain-containing protein 3; TM6SF2, transmembrane 6 superfamily 2.

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Recently, Abul-Husn et al. described a common loss-of-function variant in *HSD17B13* (rs72613567:TA) that conferred a substantial protection from chronic liver disease.[12] In an exome-wide association study of 46,544 Americans, the variant was associated with reduced plasma levels of alanine transaminase (ALT), a biochemical marker of liver cell damage.[12] In three independent case-control cohorts of liver patients of primarily European and Hispanic descent, the variant was found to be associated with lower risk of cirrhosis and hepatocellular carcinoma, by 17% to 42% in heterozygotes, and by 30% to 73% in homozygotes.[12] The hepatoprotective effect of the variant has since been confirmed in a cohort of Argentinians with histologically verified chronic liver disease,[13] in European-ancestry patients with biopsy-proven nonalcoholic fatty liver disease,[14] and in a study of French and Belgian patients with alcoholic liver disease.[15] Intriguingly, the TA-variant confers protection from the later stages of fatty liver disease, but not from the development of hepatic steatosis *per se*.[12] *HSD17B13* is expressed primarily in the liver and encodes hydroxysteroid 17-beta dehydrogenase 13, a protein of unknown function that is located on the surface of hepatic fat droplets.[12,16,17] The hepatoprotective *HSD17B13*-variant encodes a truncated protein that appears to lack normal function.[12] The mechanism underlying the protective effect of *HSD17B13* rs72613567:TA remains enigmatic. Moreover, it is unclear whether the variant reduces the impact of specific risk factors (e.g., obesity, alcohol, or genetic susceptibility factors), or if it exerts an overarching hepatoprotective effect.

In this study, we tested whether adiposity, a high intake of alcohol, or genetic risk of fatty liver disease influenced the hepatoprotective effect of *HSD17B13* rs72613567 in 111,612 individuals from the Danish general population.

Patients and Methods

PARTICIPANTS

We combined two studies of the Danish general population, the Copenhagen General Population Study (CGPS) and the Copenhagen City Heart Study (CCHS), into one cohort, referred to here as the Copenhagen Cohort.[18,19] The CGPS and CCHS are prospective studies of the Danish general population initiated, respectively, in 2003-2015 and in 1976-1978, with follow-up examinations for CCHS in 1981-1983, 1991-1994, and 2001-2003. Individuals were selected based on the national Danish Civil Registration System to reflect the adult Danish population aged 20 to 100+.[10] All individuals were white and of Danish descent, as determined by the national Danish Civil Registration System. There was no overlap of individuals between the two studies. Data were obtained from a self-administered questionnaire reviewed together with an investigator on the day of attendance, a physical examination, and blood samples. Blood samples for DNA extraction and biochemical analyses were drawn on the day of enrollment in the CGPS (2003-2015) and on the day of enrollment at the CCHS examinations in 1991-1994 and 2001-2003. Studies were approved by institutional review boards and Danish ethical committees and were conducted according to the Declaration of Helsinki. Written informed consent was obtained from the participants.

ARTICLE INFORMATION:

From the [1]Department of Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [2]Department of Clinical Biochemistry; [3]The Copenhagen General Population Study, Herlev and Gentofte Hospital, Copenhagen University Hospitals, Copenhagen, Denmark; [4]The Copenhagen City Heart Study, Bispebjerg and Frederiksberg Hospital, Copenhagen University Hospitals, Copenhagen, Denmark; [5]Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Stefan Stender, M.D., Ph.D.
Department of Clinical Biochemistry KB3011
Section for Molecular Genetics
Rigshospitalet, Copenhagen University Hospital
Blegdamsvej 9, DK-2100 Copenhagen, Denmark
E-mail: stefan.stender@regionh.dk
Tel.: +45-3545-4159

GENOTYPING

Individuals in the Copenhagen Cohort were genotyped for the loss-of-function variant (rs72613567:TA) in *HSD17B13* using a TaqMan assay (ABI PRISM 7900HT Sequence Detection System, Applied Biosystems, Foster City, CA). Genotyping was performed in 384-well format using the manufacturer's instructions. The primer and probe sequences were as follows: Forward: 5′-GCTCTATTGGTGTTTTAGTATTTGGGTGTT-3′; Reverse: 5′-AGAAGTCTGATAGATGGAATACTTACCAATAAGAA-3′; Vic-labeled probe: 5′-CTGTGCTGTACTTACTTCT-3′; and Fam-labeled probe: 5′-TGCTGTACTTAACTTCT-3′. Rs72613567 introduces an adenine at a splice site in *HSD17B13* (GRCh38.p7: NC_000004.12:g.87310240_87310241insA), causing the production of a messenger RNA transcript that encodes a truncated form of the HSD17B13 protein.[12] The variants *PNPLA3* p.I148M (rs738409), *TM6SF2* p.E167K (rs58542926), *MBOAT7* (rs641738), and *GCKR* p.P446L (rs1260326) were previously TaqMan-genotyped in the same cohort, with genotypes available in 104,997 to 111,498 individuals.[10,18,20]

LABORATORY ANALYSES

Plasma levels of ALT, albumin, alkaline phosphatase, activated partial thromboplastin time, aspartate transaminase, bilirubin, gamma glutamyltransferase, high-sensitivity C-reactive protein, and international normalized ratio were measured using standard hospital assays (Konelab, Helsinki, Finland; ACL-Top, Instrumentation Laboratory, Kirchheim, Germany; and Boehringer Mannheim, Mannheim, Germany).[20]

LIVER DISEASE OUTCOMES

Diagnoses of cirrhosis were collected from the national Danish Patient Registry and the national Danish Causes of Death Registry from January 1, 1977, to April 5, 2018. The National Danish Patient Registry has information on all patient contacts with all clinical hospital departments in Denmark, including emergency wards and outpatient clinics (from 1994). The national Danish Causes of Death Registry contains data on the causes of all deaths in Denmark, as reported by hospitals and general practitioners. Cirrhosis cases were defined as individuals with International Classification of Diseases (ICD) codes ICD-10 K70.3 (alcoholic cirrhosis, n = 246), K74.6 (unspecified cirrhosis of liver, n = 166) and/or ICD-8 codes 57109 (alcoholic cirrhosis, n = 48), 57192 (unspecific cirrhosis, n = 24), or 57199 (cirrhosis of nonalcoholic causes, n = 13). We also divided cirrhosis cases into "alcoholic cirrhosis" (K70.3 or 57109) and "other cirrhosis" (K74.6, 57912, or 57199).

Hepatocellular carcinoma cases were defined as individuals with an ICD-10 code of C22.0 (liver cell carcinoma, n = 80), C22.9 (malignant neoplasm of liver, not specified as primary or secondary, n = 18), and/or an ICD-7 code of 1550 (liver cancer, n = 0) in the Danish Cancer Registry. The Danish Cancer Registry contains information on cancer events in Denmark since 1943.[21] Of the cancer events in the entire registry, 89% have been validated histologically; from 1978 onward, this increased to nearly 100%. We included cases of hepatocellular carcinoma recorded between 1943 and December 31, 2016 (our last update of the registry). From January 1, 2017, to April 5, 2018, hepatocellular carcinoma was collected from the national Danish Patient Registry using the same ICD codes (C22.0 [n = 13], C22.9 [n = 2]). Individuals that received any of these described ICD codes in the Danish Causes of Death Registry were defined as having died from cirrhosis and/or hepatocellular carcinoma.

OTHER COVARIATES

Body mass index (BMI) was measured weight in kilograms divided by measured height in meters squared. Alcohol consumption was self-reported intake of alcohol in units per week (1 unit = 12 g of alcohol). Diabetes mellitus was defined as individuals with one or more of the following ICD codes in the national Danish Patient Registry: ICD-10 E10, E11, E13, or E14 and/or ICD-8 249 or 250, and/or with a baseline nonfasting glucose measurement of more than 11 mmol/L.

STATISTICAL ANALYSIS

All analyses were performed using R statistical software version 3.4.1. A two-sided *P* value less than 0.05 was considered significant. For statistical tests, *HSD17B13* rs72613567 genotypes were coded 0, 1, and 2 for common homozygotes (T/T), heterozygotes (T/TA), and rare homozygotes (TA/TA), respectively.

Differences in baseline characteristics among individuals with different *HSD17B13* genotypes were tested with a χ^2-test for binary traits and a Kruskal-Wallis rank sum test for continuous traits. The associations between *HSD17B13* genotype and plasma markers and liver disease outcomes were tested with linear and logistic regression, respectively. All regressions were adjusted for sex and age. Due to skewed distributions, all plasma measurements (except albumin) were natural logarithmically transformed before entering regressions. Risk of all-cause or liver-related death as a function of *HSD17B13* genotype was evaluated by Cox regression (adjusted for sex and with age as the time scale). Cumulative incidences were calculated using Aalen-Johansen estimates. For analyses of liver-related death, death due to other causes was entered as a competing risk in the model. Individuals were followed prospectively from age at study entry or from age at cirrhosis diagnosis until death or end of follow-up, whichever occurred first.

We tested for interaction between *HSD17B13* rs72613567 and BMI, alcohol consumption, sex, diabetes, steatogenic alleles, and a combined risk score on ALT by the inclusion of an interaction term between *HSD17B13* genotype and each of the covariates mentioned (one at a time) in the linear regression models. *HSD17B13* genotype and each covariate were entered as continuous variables (i.e., all tests for interaction are 1 degree of freedom). An inherent feature of quantitative interactions (i.e., interactions that affect the magnitude, but not the direction of an association) is that they depend on the scale on which they are analyzed.[22] To assess the effects of scale transformations, we repeated all interaction tests using untransformed, logarithmically transformed, exponentially transformed, or inverse normalized values of ALT.[23] The *P* values for interaction reported in the main manuscript were based on logarithmically transformed values of ALT. To account for potentially higher variance in ALT in the extreme groups of BMI, alcohol consumption, or steatogenic genetic risk (heteroscedasticity), we repeated all interaction tests using a heteroscedasticity-robust model.[24] We also tested for interaction between *HSD17B13* genotype and BMI, alcohol, and steatogenic alleles on logarithmically transformed aspartate transaminase, alkaline phosphatase, or gamma glutamyltransferase.

To depict potential interaction between *HSD17B13* genotype and risk factors for liver disease visually, we combined individuals into groups based on their BMI, alcohol consumption, and genetic susceptibility to fatty liver disease. Each risk factor was divided into four groups. BMI was divided into lean (<25 kg/m^2), overweight (25-29.9 kg/m^2), obese (30-35 kg/m^2), and very obese (>35 kg/m^2). Alcohol consumption was divided into light (<84 g per week), light-moderate (84-167 g and 84-251 g per week for women and men, respectively), moderate (168-504 g and 252-504 g per week for women and men, respectively), or heavy (>504 g per week). The total number of *PNPLA3* p.I148M and *TM6SF2* p.E167K steatogenic alleles was calculated for each individual (range: 0-4) and divided into the four groups as 0, 1, 2, and 3-4 steatogenic alleles. Full covariate data were available in 103,990 individuals from the Copenhagen Cohort. For each individual, a combined risk score (range: 0-10) was calculated as the total sum of BMI group (score: 0-3), alcohol consumption group (score: 0-3), and number of steatogenic alleles (score: 0-4). Individuals with a risk score of 6-10 were combined into one group due to very few individuals in each of these extreme groups.

Results

BASELINE CHARACTERISTICS AND GENOTYPING

The baseline characteristics of the 111,612 individuals from the Copenhagen Cohort are given in Table 1. The Copenhagen Cohort currently includes 497 individuals with a diagnosis of cirrhosis and 113 with hepatocellular carcinoma. The baseline characteristics of individuals with these diagnoses are provided in Supporting Table S1. Genotyping of *HSD17B13* rs72613567 identified 53,376 T/T homozygotes

TABLE 1. Baseline Characteristics of the Copenhagen Cohort

N	111,612
Men (%)	50,070 (45%)
Age (years)	58 (48-68)
BMI (kg/m^2)	26 (23-28)
Diabetes mellitus (%)	6,469 (6%)
Alcohol consumption (g per week)	96 (36-180)

Note: Values are numbers (and percentages) for categorical traits, or medians (and interquartile ranges) for continuous traits.

(47.8%), 47,552 T/TA heterozygotes (42.6%), and 10,684 TA/TA homozygotes (9.6%), corresponding to a TA-allele frequency of 30.9% in this Danish population. Genotype frequencies did not deviate from the Hardy-Weinberg equilibrium (P = 0.53). Sex, age, BMI, diabetes, and alcohol consumption did not differ among individuals with different *HSD17B13* genotypes (Supporting Table S2).

HSD17B13 AND BIOCHEMICAL MARKERS OF LIVER DISEASE

The liver-protective effect of *HSD17B13* rs72613567:TA was discovered based on its association with reduced plasma levels of ALT in American cohorts.[12] We validated the association with reduced ALT in the Copenhagen Cohort (Fig. 1). Each copy of the TA-allele reduced the mean plasma levels of ALT in a dose-dependent manner (P value for trend across genotypes = 2×10^{-24}). The absolute change in mean ALT between T/T homozygotes and TA/TA homozygotes was 1.3 U/L (relative change: 5.6%). The association of the TA-allele with ALT was more pronounced in men than in women (Supporting Fig. S1, P value for interaction between *HSD17B13* genotype and sex on ALT = 0.001). The TA-allele was also associated with slightly lower levels of plasma aspartate transaminase, gamma-glutamyltransferase, and bilirubin, but not with alkaline phosphatase, albumin, international normalized ratio, activated partial thromboplastin time, or high-sensitive C-reactive protein (Supporting Table S3).

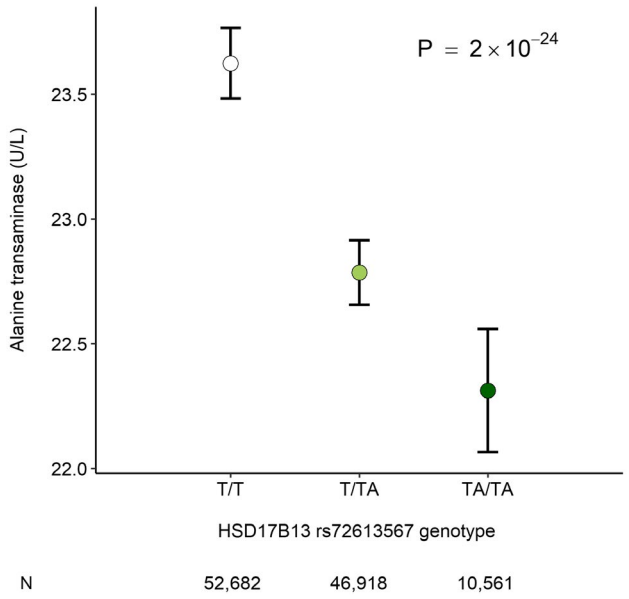

FIG. 1. Plasma ALT as a function of *HSD17B13* rs72613567 genotype. Estimates depict mean ALT, and error bars are 95% CIs. P value by linear regression is adjusted for sex and age. Note that the y-axis has been truncated for visual clarity.

HSD17B13 AND PROTECTION FROM LIVER DISEASE

Next, we analyzed the association between rs72613567 and cirrhosis and/or hepatocellular carcinoma in the Copenhagen Cohort (Fig. 2). For each TA-allele, the risk of cirrhosis and/or hepatocellular carcinoma was reduced by 17% (odds ratio [OR]: 0.83; 95% confidence interval [CI], 0.73-0.95), cirrhosis separately by 15% (OR: 0.85; 95% CI, 0.74-0.98), and hepatocellular carcinoma separately by 28% (OR: 0.72; 95% CI, 0.53-0.97). The protective effect on cirrhosis conferred by the TA-allele was similar for alcohol-related cirrhosis and for cirrhosis due to other causes.

HSD17B13 AND REDUCED LIVER-RELATED MORTALITY

We tested whether *HSD17B13* rs72613567:TA protected from liver-related and all-cause mortality in individuals from the general population, and/or in patients with a cirrhosis diagnosis. Compared with T/T homozygotes, the hazard ratios for liver-related mortality in the general population were 0.67 (95% CI, 0.50-0.90) and 0.69 (95% CI, 0.41-1.17) for T/TA heterozygotes and TA/TA homozygotes, respectively (P value for trend across genotypes = 0.01) (Fig. 3A). Among patients with cirrhosis, the corresponding hazard ratios were 0.51 (95% CI, 0.32-0.81) and 0.69 (95% CI, 0.33-1.44; P value trend = 0.02) (Fig. 3B). The TA-allele also tended to associate with reduced all-cause mortality in cirrhosis patients, but not in the general population (P value trend = 0.06 and 0.28, respectively) (Supporting Fig. S2).

INTERACTION BETWEEN *HSD17B13* AND RISK FACTORS FOR FATTY LIVER

Synergistic relationships among individual risk factors play an important role in fatty liver disease. For

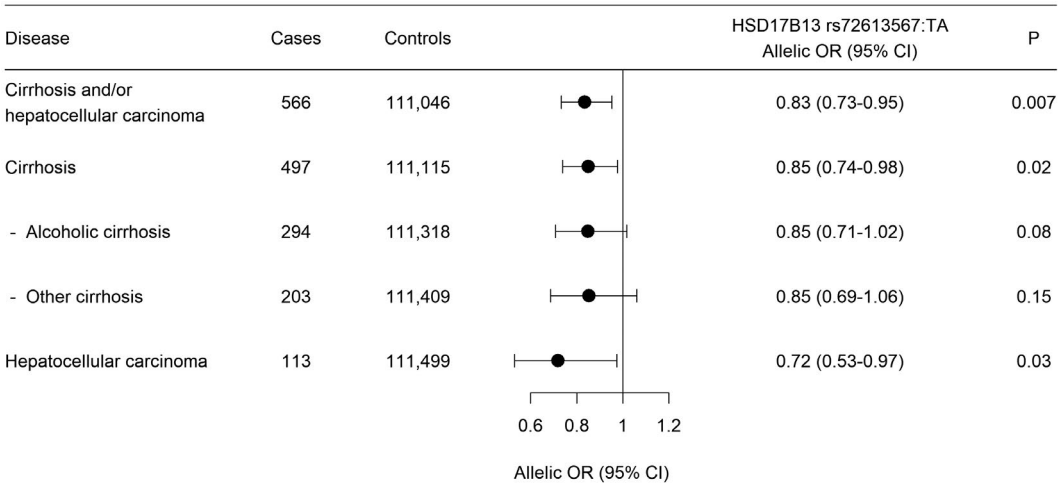

FIG. 2. Risk of liver disease as a function of *HSD17B13* rs72613567:TA allele. ORs and *P* values by logistic regression are adjusted for sex and age.

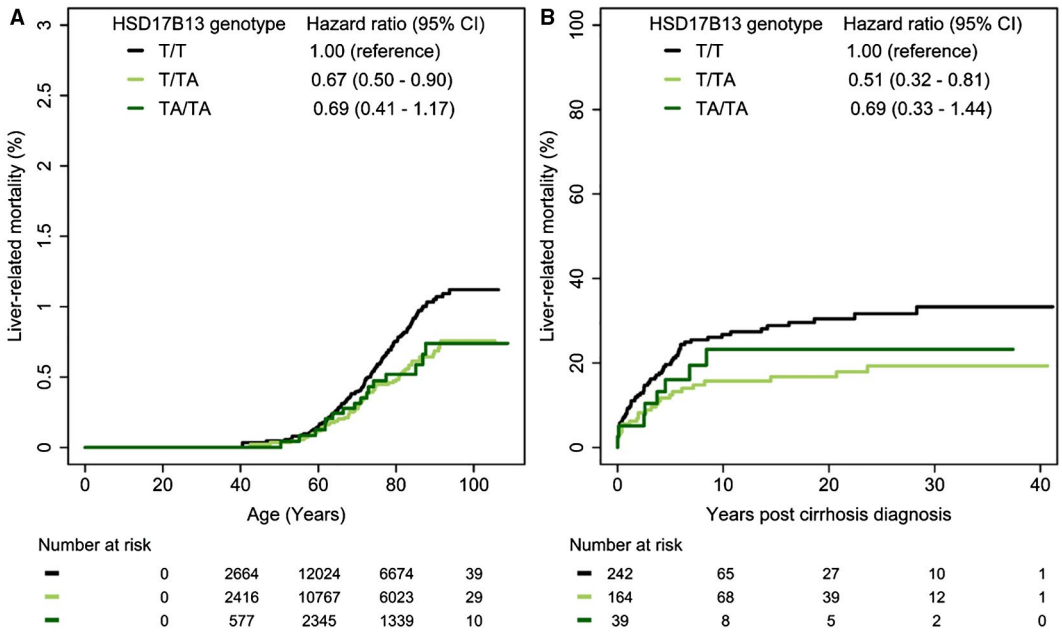

FIG. 3. Prospective liver-related mortality as a function of *HSD17B13* rs72613567 genotype. Liver-related mortality was death due to cirrhosis or hepatocellular carcinoma. (A) Liver-related mortality in the general population as a function of *HSD17B13* genotype. Participants were followed prospectively from the time of study entry until death or end of follow-up. (B) Liver-related mortality in patients with cirrhosis as a function of *HSD17B13* genotype. Participants were followed prospectively from the time of cirrhosis diagnosis until death or end of follow-up. Cumulative incidences are Aalen-Johansen estimates, adjusted for death due to other causes. Hazard ratios were calculated by Cox regression, adjusted for sex and with age as the underlying time scale.

example, obesity amplifies the genetic risk of fatty liver, as well as the hepatotoxic effect of alcohol.[18,25] We wondered whether similar synergistic effects modify the ALT-lowering effect of *HSD17B13* rs72613567.

Obesity

In the Copenhagen Cohort, the ALT-lowering effect of the TA-allele was amplified by higher BMI (*P* value

for interaction between rs72613567 and BMI on ALT = 0.003) (Fig. 4, left panel). In lean individuals with a BMI below 25 kg/m^2, mean plasma ALT was 20.0 U/L in T/T homozygotes and 19.3 U/L in TA/TA homozygotes (absolute difference: 0.7 U/L, relative difference: 3.5%). Among the most obese individuals with a BMI above 35 kg/m^2, the mean ALT was 31.3 U/L in T/T homozygotes and 27.6 U/L in TA/TA homozygotes (absolute difference: 3.7 U/L, relative difference: 11.8%).

Diabetes

The presence of diabetes at baseline augmented the ALT-lowering effect of the TA-allele (Supporting Fig. S3, *P* value for interaction between rs72613567 and diabetes on ALT = 0.02). Among nondiabetics, the mean plasma ALT was 23.2 U/L in T/T homozygotes and 22.0 U/L in TA/TA homozygotes (absolute difference: 1.2 U/L, relative difference: 5.3%). The corresponding values in individuals with diabetes were 29.7 U/L and 27.3 U/L (absolute: 2.4 U/L, relative: 8.1%).

Alcohol Consumption

A similar amplification of the ALT-lowering effect of the TA-allele was observed with higher alcohol consumption (*P* value for interaction between rs72613567 and alcohol consumption on ALT = 3 × 10^{-5}) (Fig. 4, middle panel). In individuals with light alcohol consumption, the absolute difference in ALT was 1.0 U/L (relative difference: 4.6%) for T/T homozygotes versus TA/TA homozygotes. The corresponding difference in heavy drinkers was 6.5 U/L (relative: 18.4%).

Genetic Risk of Fatty Liver

We evaluated four variants that have previously been associated with fatty liver disease and/or cirrhosis: *PNPLA3* p.I148M, *TM6SF2* p.E167K, *MBOAT7*

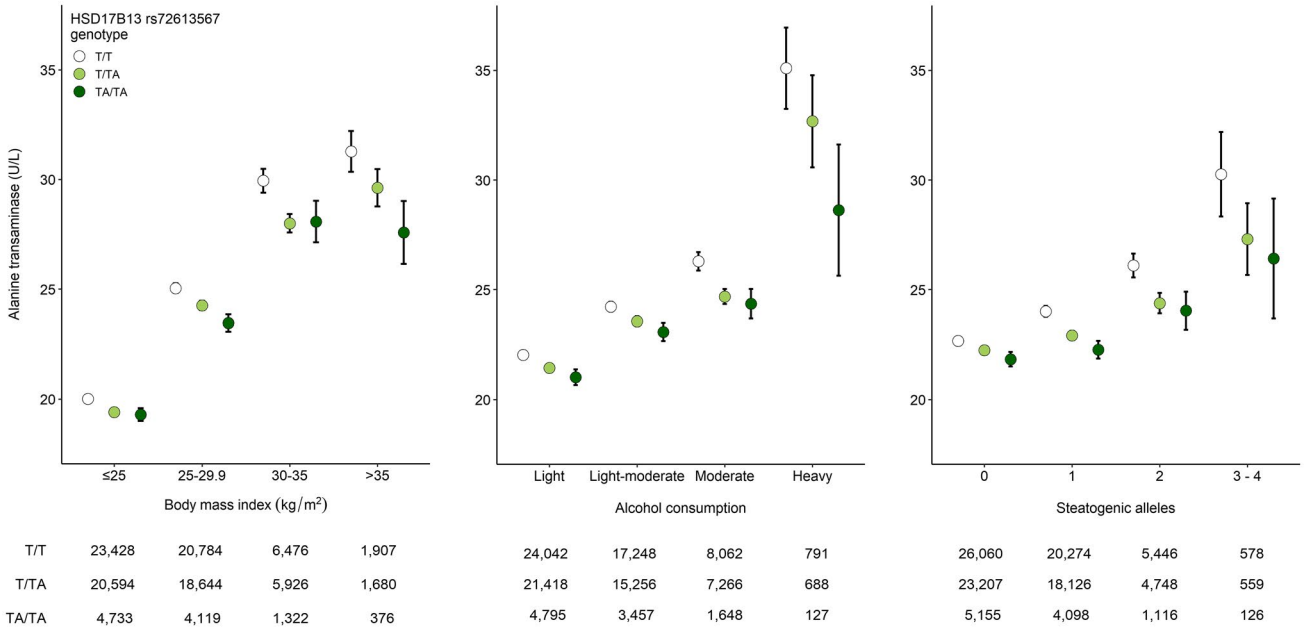

FIG. 4. Plasma ALT as a function of BMI, alcohol consumption, or genetic susceptibility to fatty liver disease, stratified by *HSD17B13* rs72613567 genotype. The estimates depict mean ALT, and the error bars are 95% CIs. Note that the y-axis has been truncated for visual clarity. BMI was grouped into lean (<25 kg/m^2), overweight (25-29.9 kg/m^2), obese (>30-35 kg/m^2), and very obese (>35 kg/m^2). Alcohol consumption was grouped into light (<84 g per week), light-moderate (84-167 g and 84-251 g per week for women and men, respectively), moderate (168-504 g and 252-504 g per week for women and men, respectively), and heavy (>504 g per week). Genetic susceptibility to fatty liver disease was calculated by counting the total number of steatogenic alleles in *PNPLA3* p.I148M and *TM6SF2* p.E167K for each individual (range: 0-4). The ALT-lowering effect of the rs72613567 TA-allele was amplified by increasing adiposity, alcohol intake, or genetic susceptibility to fatty liver (*P* value for interaction: 0.003, 3 × 10^{-5}, and 0.01, respectively).

rs641738, and *GCKR* p.P446L.[8,10,26-28] Of these, only the *PNPLA3* and *TM6SF2* variants were associated with higher plasma ALT, and with an increased risk of cirrhosis or hepatocellular carcinoma in our cohort (Supporting Table S4). We combined these two variants into a genetic risk score by counting the total number of steatogenic alleles for each participant. An increasing genetic risk score amplified the ALT-lowering effect of *HSD17B13* (*P* value for interaction between rs72613567 and steatogenic alleles *PNPLA3* p.I148M and *TM6SF2* p.E167K = 0.01) (Fig. 4, right panel). In individuals with zero steatogenic alleles in *PNPLA3* and *TM6SF2*, the absolute difference in mean ALT between T/T homozygotes and TA/TA homozygotes was 0.8 U/L (relative difference: 3.7%). The corresponding difference was 3.8 U/L (relative: 12.7%) among those with three or four steatogenic alleles. The *HSD17B13* variant interacted with *PNPLA3* p.I148M when viewed in isolation (*P* value for interaction = 0.004), but not with *TM6SF2* p.E167K (*P* = 0.69) on ALT (Supporting Fig. S4).

Combined Risk Score

We combined adiposity, alcohol consumption, and genetic risk of fatty liver into a risk score (range 0-10). Similar to what we observed for the individual risk factors, the ALT-lowering effect of rs72613567:TA was amplified with increasing combined risk score (*P* value for interaction between rs72613567 and risk score on ALT = 6×10^{-7}) (Fig. 5). In individuals with a risk score of zero, the absolute difference in ALT was 0.2 U/L between T/T homozygotes and TA/TA homozygotes (relative: 1.2%). The corresponding difference was 7.3 U/L (relative difference: 17.2%) among individuals with a risk score of 6 to 10.

SENSITIVITY ANALYSIS

Synergistic effects on continuous traits are influenced by the scale on which they are analyzed.[23] We therefore repeated the tests for interaction between *HSD17B13* and fatty liver risk factors on ALT using either untransformed, exponentiated, or inverse normalized values of ALT. To account for possible effects of heteroscedasticity, we also repeated the same interaction tests using model-robust estimates of standard errors.[24] The interactions between *HSD17B13* and the individual fatty liver risk factors on ALT remained, regardless of transformation or model applied (Supporting Fig. S5). There were modest interactions (*P* = 0.01 to 0.03) between *HSD17B13* and BMI, alcohol intake, or steatogenic alleles on aspartate transaminase, alkaline phosphatase, and bilirubin, but not on gamma-glutamyltransferase (Supporting Table S5).

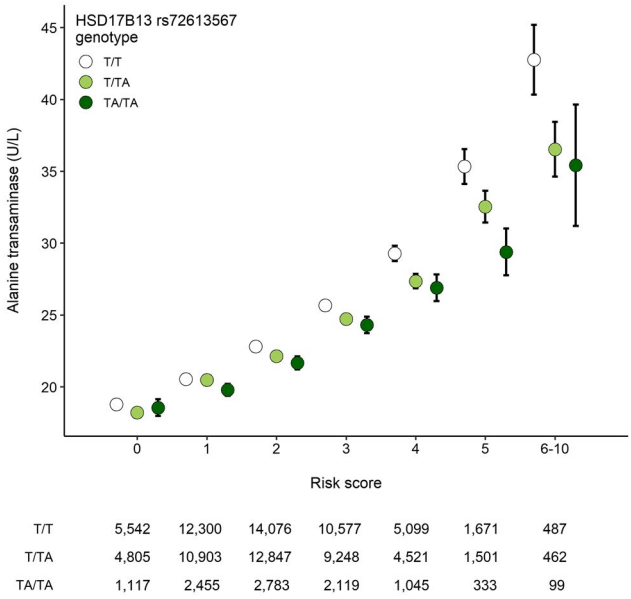

FIG. 5. Plasma ALT as a function of combined risk score, stratified by *HSD17B13* rs72613567 genotype. The estimates depict mean ALT, and the error bars are 95% CIs. Note that the y-axis has been truncated for visual clarity. The combined risk score (range 0-10) was calculated as the sum of the risk factors of adiposity (lean = 0, overweight = 1, obese = 2, very obese = 3), alcohol consumption (light = 0, light-moderate = 1, moderate = 2, heavy = 3), and the number of steatogenic alleles in *PNPLA3* p.I148M and *TM6SF2* p.E167K (range 0-4) for each individual. The ALT-lowering effect of the rs72613567 TA-allele was amplified by the increasing risk score (*P* value interaction between rs72613567 and risk score on ALT = 6×10^{-7}).

Discussion

The main finding of this study is that the ALT-lowering effect of a common loss-of-function variant in *HSD17B13* is amplified by high risk of fatty liver disease. Increasing adiposity, alcohol intake, as well as genetic risk of hepatic steatosis progressively unmasked the association of *HSD17B13* rs72613567:TA with reduced plasma levels of ALT in 111,612 persons

from the Danish general population. The variant was associated with only marginally lower ALT in lean nondrinkers with low genetic risk of hepatic steatosis, whereas homozygosity for the protective TA-allele was associated with 12% to 18% lower plasma ALT in the most obese, in heavy drinkers, and in individuals carrying three or four steatogenic alleles in *PNPLA3* and *TM6SF2*. The findings are in line with previous studies that reported gene–environment interactions affecting fatty liver disease.[18,29-31] Just as the M-allele of *PNPLA3* p.I148M has a greater harmful effect in the most obese, the TA-variant of *HSD17B13* appears to have the greatest protective effect in high-risk individuals.

Loss-of-function genetic variants that protect from disease are interesting, because they pinpoint potential drug targets.[32] Therapeutic inhibition of HSD17B13 is already being explored by the pharmaceutical industry, aiming to recapitulate the protection from liver disease seen in carriers of loss-of-function *HSD17B13* variants. The data reported in the present study lend support to the notion that HSD17B13 inhibition might be particularly beneficial in those at highest risk of liver disease.

The hepatoprotective effect of *HSD17B13* rs72613567:TA was first described by Abul-Husn et al. in 2018.[12] In an exome-wide association study of 46,544 individuals, the TA-allele of rs72613567 was found to be associated with lower plasma levels of ALT and aspartate transaminase. The ALT-lowering effect of rs72613567:TA was most pronounced among carriers of *PNPLA3* p.I148M.[12] The variant was subsequently shown to reduce the risk of chronic liver disease by up to 73% in case-control cohorts of obese individuals of primarily white and Hispanic American ancestry.[12] We replicated the association between rs72613567 and lower levels of plasma ALT and aspartate transaminase in the Danish general population. Moreover, we found the TA-allele to associate with slightly lower levels of plasma gamma-glutamyltransferase and bilirubin, but not with alkaline phosphatase or albumin. Elevated alkaline phosphatase can reflect cholestatic injury, whereas low serum albumin may be indicative of liver failure. Cholestasis and/or liver failure are associated with advanced stages of cirrhosis. The lack of association between the TA-variant and alkaline phosphatase or albumin in our general population cohort may reflect that few individuals with advanced liver disease are likely to show up at the baseline examination (when blood for biochemical analyses was drawn).

We also replicated the association between the TA-allele and reduced risk of chronic liver disease. However, the risk reductions we observed (15%-28% per TA-allele) were slightly attenuated compared with those reported by Abul-Husn et al. (23%-44%). Potential explanations for this discrepancy include differences in population characteristics (primarily normal-weight Danes versus Americans with obesity), study design (general population versus case control), and disease definition (ICD code versus histologically verified outcomes). An observation in the present study is that the TA-allele protected from liver-related mortality, both in the general population and among patients with cirrhosis. Compared with noncarriers, T/TA heterozygotes had a 33% lower risk of dying from cirrhosis or hepatocellular carcinoma in the general population. Among cirrhosis patients, the corresponding risk reduction was 49%. The risk reductions for TA/TA homozygotes were similar, but the CIs for these estimates were wide and overlapped zero.

The biological function of HSD17B13 and the mechanism by which loss of *HSD17B13* protects from liver disease remain unclear. HSD17B13 is a member of the short-chain dehydrogenase/reductase family, a group of enzymes involved in the metabolism of steroid hormones, prostaglandins, lipids, xenobiotics, and retinoids.[16,33] The HSD17B13 protein is 300 amino acids long and contains an NAD(P)H binding site, a catalytic site, and a lipid droplet binding domain that targets the protein to the surface of hepatic lipid droplets.[14] Studies of mice that either lack or overexpress *HSD17B13* have reported conflicting results. Adam et al. found that *hds17b13* knockout mice developed hepatic steatosis and steatohepatitis due to increased hepatic *de novo* lipogenesis.[34] Paradoxically, another study found that adenoviral overexpression of *HSD17B13* in mice promoted the development of hepatic steatosis through increased sterol regulatory element binding protein 1c activity.[35] In other words, knockout as well as overexpression of *HSD17B13* has been linked to increased hepatic steatosis in mice. In contrast, *HSD17B13* rs72613567 has not been associated with altered liver fat content in humans.[12,14] The degree to which the phenotypic effects of *hds17b13* perturbation in mice resemble those seen in human carriers of *HSD17B13* mutations therefore remains unclear. Ma et al. recently showed that HSD17B13

catalyzes the oxidation of retinol to retinaldehyde, the rate-limiting step in all-trans retinoic acid synthesis.[14] Retinol plays a key role in the activation of hepatic stellate cells into fibrogenic myofibroblasts, cells that promote the development of hepatic fibrosis.[36] It is possible that HSD17B13 influences the risk of chronic liver disease via effects on hepatic retinol metabolism and stellate cell activity.

Fatty liver disease is more common in Hispanics than in blacks and whites.[1] This difference is partly due to the high frequency of the steatogenic M-allele of *PNPLA3* p.I148M among Hispanics.[8] Like *PNPLA3* p.I148M, the frequency of the hepatoprotective TA-allele of rs72613567 varies by ethnicity. It is most common in East Asians (allele frequency 27%-40%) and Europeans (22%-31%), and less common in Hispanic Americans (9%) and Africans (1%-8%). Another hepatoprotective loss-of-function variant in *HSD17B13* was recently described (rs143404524, p.Ala192LeufsTer8).[37] This variant is common in black Americans (19%), but rare in white and Hispanic individuals (<3%). Similar to what has been shown for *PNPLA3* p.I148M, varying frequencies of *HSD17B13* mutations likely account for some of the interethnic differences in susceptibility to fatty liver disease.[8]

Our study has limitations that merit consideration. We only studied white individuals of European descent. Our findings may therefore not be generalizable outside Western populations. Another limitation is that outcomes based on ICD codes received in hospitals are likely to suffer from some degree of misclassification. However, the validity of the ICD-defined cirrhosis endpoint used here is supported by the fact that *PNPLA3* p.I148M was strongly associated with it,[20] with per-allele ORs comparable to those reported in other studies that used histology.[38] Misclassification was likely very low for the hepatocellular carcinoma cases, because these were extracted from the Danish Cancer Register, a register that primarily includes histologically validated cancers.[21] We did not have available liver tissue from the individuals, nor detailed information on the etiology underlying the clinical outcomes. Finally, detection of statistical interaction requires comprehensive power (at least 4 times that needed to detect a main effect of similar magnitude).[39] The number of individuals with ICD-defined cirrhosis and hepatocellular carcinoma in the cohort is still too low to allow for meaningful tests of interaction on these clinical endpoints. However, we were able to show robust interactions between *HSD17B13* and adiposity, alcohol, and genetic steatogenic risk on plasma ALT, a biochemical marker of liver disease.

In summary, these results indicate that in the Danish general population, the ALT-lowering effect of the loss-of-function variant *HSD17B13* rs72613567:TA is most pronounced among those at highest risk of fatty liver disease.

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Author names in bold designate shared co-first authorship.

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