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*ADH1B*2* is Associated With Reduced Severity of Nonalcoholic Fatty Liver Disease in Adults, Independent of Alcohol Consumption

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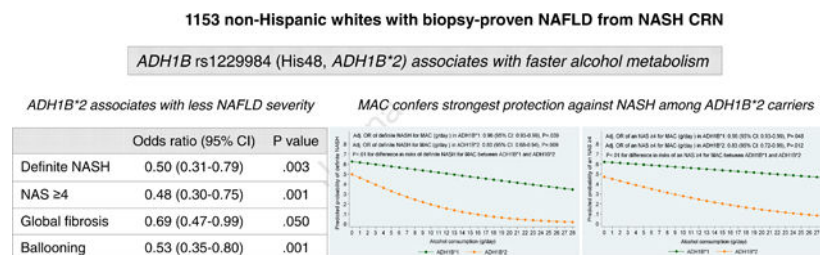
Abstract

Background & Aims: Alcohol dehydrogenase 1B (ADH1B) is involved in alcohol metabolism. The allele A (*ADH1B*2)* of rs1229984: A>G variant in *ADH1B* is associated a higher alcohol metabolizing activity, compared to the ancestral allele G (*ADH1B*1*). Moderate alcohol consumption is associated with reduced severity of nonalcoholic fatty liver disease (NAFLD), based on histologic analysis, compared with no alcohol consumption. However, it is unclear whether *ADH1B*2* modifies the relationship between moderate alcohol consumption and severity of NAFLD. We examined the association between *ADH1B*2* and moderate alcohol consumption and histologic severity of NAFLD.

Methods: We collected data from 1557 multi-ethnic adult patients with biopsy-proven NAFLD enrolled into 4 different studies conducted by the NASH Clinical Research Network. Histories of alcohol consumption were obtained from answers to standardized questionnaires. Liver biopsies were analyzed by histology and scored centrally according to the NASH CRN criteria. We performed covariate adjusted logistic regressions to identify associations between histologic features of NAFLD severity and moderate alcohol consumption and/or *ADH1B*2.*

Results: A higher proportion of Asians/Pacific Islanders/Hawaiians carried the *ADH1B*2* allele (86%) than other racial groups (4%–13%). However, the study population comprised mostly non-Hispanic whites (1153 patients, 74%), so the primary analysis focused on this group. Among them, 433 were moderate drinkers and 90 were *ADH1B*2* carriers. After we adjusted for confounders, including alcohol consumption status, *ADH1B*2* was associated with lower frequency of steatohepatitis (odds ratio [OR], 0.52; *P*<.01) or fibrosis (odds ratio, 0.69; *P*=.050) compared with *ADH1B*1*. Moderate alcohol consumption (g/day) reduced the severity of NAFLD in patients with *ADH1B*1* or *ADH1B*2*. However, *ADH1B*2*, compared to *ADH1B*1*, was associated with a reduced risk of definite NASH (*ADH1B*2* OR, 0.80; *P*<.01 vs *ADH1B*1* OR, 0.96; *P*=.036) and a reduced risk of an NAFLD activity score of 4 or higher (*ADH1B*2* OR, 0.83; *P*=.012 vs *ADH1B*1* OR, 0.96; *P*=.048) (*P*<.01 for the difference in the effect of moderate alcohol consumption between alleles). The relationship between body mass index and NAFLD severity was significantly modified by *ADH1B*2*, even after we controlled for alcohol consumption.

Conclusions: *ADH1B*2* reduces the risk of NASH and fibrosis in adults with NAFLD regardless of alcohol consumption status. *ADH1B*2* might modify the association between high body mass index and NAFLD severity.

Graphical Abstract

1153 non-Hispanic whites with biopsy-proven NAFLD from NASH CRN

ADH1B rs1229984 (His48, ADH1B*2) associates with faster alcohol metabolism

ADH1B*2 associates with less NAFLD severity

	Odds ratio (95% CI)	P value
Definite NASH	0.50 (0.31-0.79)	.003
NAS ≥4	0.48 (0.30-0.75)	.001
Global fibrosis	0.69 (0.47-0.99)	.050
Ballooning	0.53 (0.35-0.80)	.001

MAC confers strongest protection against NASH among ADH1B*2 carriers

Keywords

progression; risk factor; outcome; histology

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is currently the most common cause of chronic liver disease (CLD), affecting up to one third of American adults.[1] By definition, a diagnosis of NAFLD requires the exclusion of significant alcohol consumption, which means > 7 and > 14 standard drinks per week for women and men, respectively.[2] It is worth to mention that up to 40% of patients with NAFLD report lifetime history of moderate alcohol consumption (MAC).[3] The effect of MAC in patients with NAFLD remains controversial, with some cross-sectional studies reporting that a MAC is associated with less severity in NAFLD and fibrosis.[3–5] Over the past decade, several longitudinal studies have shown that MAC lowers the risk of type 2 diabetes mellitus (T2DM), hypertension, cardiovascular events, and these benefits may be partly explained by increased insulin sensitivity and high-density lipoprotein (HDL) levels as well as favorable effects on homeostatic factors and inflammatory pathways.[6–8]

Alcohol dehydrogenase (ADH) enzymes are key regulators of alcohol metabolism. Polymorphisms in the genes encoding these enzymes result in the production of enzymes with different kinetic properties and different alcohol oxidizing capacities.[9] One of the most studied single-nucleotide polymorphism in the *ADH1B* (Alcohol Dehydrogenase 1B (class I), Beta Polypeptide) gene is rs1229984, a G to A base transition in exon 3 leading to the substitution of arginine (Arg48, *ADH1B*1*) to histidine (His48, *ADH1B*2*) at position 48. *ADH1B*2* allele encodes for an enzyme with approximately 80-fold higher turnover rate and 40 times more activity in producing acetaldehyde than *ADH1B* Arg48.[9] Acetaldehyde buildup in the blood is associated with many unpleasant reactions following the consumption of alcohol, thus individuals with *ADH1B*2* allele may refrain from drinking large quantities of alcoholic beverages and, therefore, may be protected against alcohol use disorders.[10] However, whether this unpleasant reaction to alcohol in *ADH1B*2* carriers results in a reluctance to drink alcohol after light or moderate alcohol intake is unclear. To address this question, a recent cross-sectional study assessed the effect of *ADH1B*2* allele on the amount of alcohol consumption as well as its association with liver histology severity among patients with biopsy confirmed NAFLD.[11] By using a Mendelian randomization analysis, authors found that carriers of the *ADH1B*2* allele not only had lower consumption of alcohol but also decreased scores of histological steatosis, lobular inflammation and NAFLD activity score. The authors of the study suggest that the effect of *ADH1B*2* allele on the liver histology may be mediated exclusively by the amount of alcohol consumed and not by any other biological effect.

The above-mentioned findings provided us the rationale to explore the relationship between MAC, the presence of *ADH1B*2* allele and the liver histology severity in a large cohort of patients with biopsy-proven NAFLD. Thus, our study is aimed to determine the impact of *ADH1B* alleles on the current or lifetime average of alcohol intake, and to identify potential effects of current history of MAC or *ADH1B* alleles on histological severity of NAFLD. Additionally, we tested interactions effects between MAC and *ADH1B* rs1229984, and its potential effects on NAFLD histological phenotypes.

METHODS

Study design

This is a cross-sectional study of prospectively evaluated adult patients with biopsy-proven NAFLD who were enrolled into various studies conducted by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)-sponsored Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN). The present analysis includes patients enrolled into NAFLD Adult Database 1 (enrolling patients between 2004–2009), PIVENS (NCT00063622), FLINT (NCT01265498) and NAFLD Adult Database 2 (NCT01030484) (enrolling patients between 2009–2019), which is an extension of the NAFLD Adult Database 1 and uses the same patient's selection criteria.[12][13]

Briefly, individuals 18 years or older with a histological diagnosis of NAFLD were recruited at nine U.S. medical centers. Exclusion criteria included clinical or histological evidence of alcoholic liver disease or alcohol consumption of >7 and >14 standard drinks per week for women and men, respectively in the 2 years prior to screening, other causes of CLD, history of total parenteral nutrition, biliopancreatic diversion or bariatric surgery, short bowel syndrome, suspected or confirmed hepatocellular carcinoma and positivity for HIV. For the purpose of enrollment into this observational study, the diagnosis of NAFLD was based on the histological diagnosis of NAFLD or cryptogenic cirrhosis or on imaging studies. The current analysis included participants who had collected information on central review of liver histology, alcohol consumption questionnaires (AUDIT and lifetime drinking history),[14,15] *ADH1B* rs1229984 and *PNPLA3* rs738409 genotyping through July 2019.

Participants with lifetime history of binge (4 or 5 standard drinks for women and men in a typical drinking day) or heavy drinking (>7 and >14 standard drinks per week for women and men, respectively) as captured by Lifetime Drinking History (LDH) questionnaire were excluded.[15]

All patients gave written informed consent. The medical ethical committees of the participating hospitals approved the study protocol.

Assessment of liver histology

For this study, only patients with liver biopsy data available within 6 months of the clinic data were included. The NASH CRN Pathology committee, constituted by a group of nine hepatopathologists who were unaware to all clinical and identifying data, reviewed all liver biopsies centrally. The histological diagnosis was based on consensus recognition of a classical zone 3 distribution of histological features including steatosis, lobular inflammation and ballooning hepatocyte, thus, diagnosis of NASH was established independent of the NAS scoring and classified as definite steatohepatitis, simple steatosis or suspicious for NASH ("borderline" NASH) based upon central pathology reading.[16] For the purposes of this analysis, the presence of definite steatohepatitis, an NAFLD activity score of 4 of higher including at least 1 point for each individual component (steatosis=1, lobular inflammation=1 and ballooning=1) and advanced stages of fibrosis (bridging fibrosis or cirrhosis) were considered as measures of NAFLD severity. Each of these three histological categories seem to be associated with higher risks of progression to cirrhosis or NAFLD-

related complications. Other study outcomes included the analysis of ordered categorical histological variables such as steatosis (<5%, 5–33%, 34–66%, >66%), lobular inflammation (<2, 2–4, and >4 × 20 field), portal inflammation (none, mild, more than mild), ballooning hepatocellular degeneration (none, few, many), Mallory-Denk bodies (absent or rare, many), and fibrosis stages (0= no fibrosis; 1= mild/zone 3 perisinusoidal fibrosis or moderate/zone 3 perisinusoidal fibrosis or portal/periportal only fibrosis; 2= zone 3 perisinusoidal and periportal fibrosis; 3= bridging fibrosis; 4=cirrhosis).[2,16,17]

Alcohol consumption assessment

At study enrollment, a comprehensive history of alcohol consumption was obtained via AUDIT[14] and LDH[15] questionnaires in all patients. For our primary analysis, the exposure of alcohol intake was classified as follows: (1) moderate drinkers defined as having a history of MAC (7 and 14 standard drinks per week for women and men, respectively) during the 2 years before study enrollment, and (2) non-drinkers defined as absence of any amount of alcohol intake during the 2 years preceding study entry. The last group included either those who did not report drinking alcohol at any time or those who had a previous history of MAC but became abstainers in the last 2 years preceding the liver biopsy. In order to quantify the daily intake of alcohol (g/day), we considered that approximately 14 g of alcohol equals one 'drink' unit. One unit equals 1 ounce of distilled spirits, one 12-oz beer, or one 4-oz glass of wine. More details on alcohol consumption can be found in the supplemental material.

ADH1B rs1229984 and PNPLA3 rs738409 genotyping

DNA samples were received from the CRN consortium at a minimum concentration of 50 ng/µl per sample. *ADH1B* (rs1229984) (NM_000668.6:c143 A>G) is a missense coding variant; this single nucleotide polymorphism results in an arginine [CGC] to histidine [CAC] substitution. SNP (rs738409) in *PNPLA3* gene is a missense coding variant as well (NM_025225.3: c.444C>G), and results in an Ile [ATC] to Met [ATG] substitution. For quality control of *ADH1B* genotyping, duplicated analyses were conducted in 96 randomly selected samples. Genotyping results were identical between duplicated samples. More detailed information can be found in supplemental material.

Clinical and laboratory parameters assessment

The following parameters were systematically assessed in all participants at the enrollment visit: demographic factors including age, sex, race, and ethnicity; anthropometrics including body mass index (BMI [kg/m^2]) and waist circumference; the presence of comorbidities including arterial hypertension and T2DM; laboratory tests including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, a lipid panel comprising total cholesterol, HDL, low-density lipoprotein (LDL) cholesterol and triglycerides, and finally hemoglobin A1c (HbA1c), fasting glucose and insulin as well as the homeostasis model assessment of insulin resistance (HOMA-IR) index.

Statistical analysis

Data are expressed as mean (SD), and number and percent. Chi-squared or Mantel-Haenszel trend tests were used for binary categorical variables. *T* test or Wilcoxon rank-sum test were used for continuous variables (e.g., to assess the association between continuous baseline characteristics and *ADH1B* alleles). The dose response association between daily alcohol consumption (0, 0–6.9, 7–13.9, and 14–28 grams per day) or *ADH1B* alleles with histological features including 2 or more ordinal categories was tested using the Cochran-Armitage trend test.

Because of the low minor allele frequency for *ADH1B*2* (A allele) and the small number of subjects homozygous for the minor allele (0.6%), the GA heterozygous and AA homozygous individuals were combined into a single group for analysis and compared with GG (*ADH1B*1* allele) homozygous individuals. An exact test was used to examine Hardy-Weinberg Equilibrium (HWE). Since *PNPLA3* and *ADH1B* are in different chromosomes, there is no need for linkage disequilibrium analysis.

Logistic regression models were used to estimate the odds ratios (ORs) and corresponding 95% confidence intervals (CIs). To assess the association between history of MAC (drinkers vs non-drinkers) and *ADH1B* alleles (*ADH1B*1* vs *ADH1B*2*) and ordinal histological categorical variables including three or more groups, multiple ordered logistic regression models were performed. Binary logistic regression analyses were performed for testing association between binary histological variables (e.g., definite NASH vs no NASH) and history of MAC and *ADH1B* alleles. Although the independence of the effect of *ADH1B* alleles on liver histology severity was explored through covariate-adjusted logistic analysis, we also sought to illustrate the effect of *ADH1B* alleles on histological outcomes among drinkers and non-drinkers, separately.

Multivariable adjusting models included age, gender, race (Non-Hispanic whites, Hispanics, Blacks, Asians/Pacific Islanders/Hawaiians and others), BMI, T2DM and *PNPLA3* rs738409 genotypes. Margin plots with its predicted probabilities for the presence of definite NASH or an NAS 4 were displayed to visualize the main effect of a one unit change in alcohol consumption (g/day) or BMI (kg/m^2) for each *ADH1B* allele and the impact on histological outcomes.[18] Measures of interaction on a multiplicative or an additive scale were used to assess interactions between alcohol consumption [yes/no] or BMI (37 or >37 kg/m^2) and *ADH1B* alleles.[19] Interaction on an additive scale was quantified using the relative excess risk due to interaction (RERI) and the proportion attributable to interaction (AP).[19] No missing observations were found for those variables included in our main statistical analyses (liver histology scores, *ADH1B* alleles and *PNPLA3* rs738409 genotypes, alcohol consumption history, BMI, age, gender and T2DM).

A 2-tailed p-value <.05 was considered significant. Statistical analyses were carried out with the Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC.

RESULTS

A total of 1697 patients were assessed for eligibility, and 1557 were included for baseline data and outcome analysis. Based on lifetime drinking history, 39 and 131 binge and heavy drinkers were excluded (Figure 1). The frequency of *ADH1B*2* carriage varied widely across race/ethnic categories, being high in Asians/Pacific Islanders/Hawaiians (86%) and low in non-Hispanic whites (8%), Hispanics (14%) and Blacks (4%). The Hardy-Weinberg test confirmed the independent segregation of the individual *ADH1B* alleles (p =.08 for Asians/Pacific Islanders/Hawaiians and p =.06 for non-Hispanic whites). The supplemental Table 1 shows baseline patients' characteristics in the entire multi-ethnic cohort (n=1557). In order to reduce the effect of race/ethnicity on *ADH1B* allelic frequencies and its potential influence on NAFLD severity, our primary analysis focuses on non-Hispanic whites who comprise 74% (n=1153) of the whole study population.

Baseline characteristics among non-Hispanic whites

According to the most recent history of MAC (during the 2 years preceding the study enrollment), the cohort included 720 non-drinkers and 433 moderate drinkers (Figure 1). The mean age is 50.73 ± 11.46 years, and most patients are females (727, 63%), obese (mean BMI, 34.87 ± 6.41) and have hypertension (685, 59%). T2DM is observed in 408 (35%) patients. Three hundred and forty-four (30%) have advanced fibrosis (stages of fibrosis 3), 689 (60%) and 690 (60%) have definite NASH or an NAS 4 on liver histology reports.

Baseline characteristics according to ADH1B alleles (ADH1B*1 vs ADH1B*2)

Table 1 displays demographical, clinical and laboratory characteristics according to *ADH1B* alleles. As compared to *ADH1B*1, ADH1B*2* carriers are more likely to be male and have higher insulin sensitivity and triglycerides levels. The proportion of patients with lifetime or current history of MAC is not different between both alleles. The daily average of current alcohol consumption (g/day) is 1.68 ± 3.63 in *ADH1B*2* as compared with 1.49 ± 3.49 in *ADH1B*1*, P=.630.

Association between alcohol consumption and liver histology severity

Table 2 shows the frequency and adjusted odds ratios for each histological feature based on the history of MAC. The prevalence of definite NASH, an NAS 4 and advanced fibrosis (stages of fibrosis 3) is significantly lower among drinkers compared with non-drinkers (52% vs 64%, 54% vs 63%, and 25% vs 33% respectively). Compared with non-drinkers, MAC is associated with a significantly decreased risk of severity of steatohepatitis (Adj. OR: 0.73 [95% CI: 0.57–0.94]), global fibrosis (Adj. OR: 0.68 [95% CI: 0.55–0.85]), definite steatohepatitis (Adj. OR: 0.70 [95% CI: 0.54–0.91]) and an NAS 4 (Adj. OR: 0.77 [95% CI: 0.60–0.99]) after adjustment by potential confounders, including *ADH1B* alleles, age, gender, BMI, T2DM and *PNPLA3* rs738409 genotypes. In further sensitivity analysis including the entire multi-ethnic cohort and adjusting by same variables plus race/ethnicities, the association of MAC with steatohepatitis and fibrosis remains statistically significant (supplemental Table 2).

A strong dose-dependent association is observed among daily MAC (g/day) and several histology features of NAFLD (Table 3). A higher amount of cumulative dose of alcohol consumed (between 1–28 g/day) is found to be associated with a lesser severity of ballooning hepatocyte degeneration, portal inflammation, Mallory-Denk bodies, steatohepatitis and fibrosis; these associations also remain significant when analyzed in the multi-ethnic cohort. (supplemental Tables 2 and 3).

Association between ADH1B alleles and liver histology severity

The presence of *ADH1B*2* allele is associated with lower severity in lobular inflammation, hepatocyte ballooning degeneration, fibrosis, and steatohepatitis including definite NASH and an NAS 4 as compared with *ADH1B*1* (Table 2). On the multivariable adjusted analyses, *ADH1B*2* carriers show reduced risk of severity of steatohepatitis (Adj. OR: 0.52 [95% CI: 0.34–0.78]) and fibrosis (Adj. OR: 0.69 [95% CI: 0.47–0.99]) as well as definite NASH (Adj. OR: 0.50 [95% CI: 0.31–0.79]) and an NAS 4 (Adj. OR: 0.48 [95% CI: 0.30–0.75]); these associations remain significant even after adjustments by alcohol consumption, gender, BMI, T2DM and *PNPLA3* rs738409 genotypes as shown in Table 2 and supplemental Tables 4 and 5. Similar associations were found in the multi-ethnic cohort as compared with the non-Hispanic white population, with the exception of lobular inflammation (supplemental Table 2).

Finally, no significant interactions were seen between *PNPLA3* rs738409 SNP and either *ADH1B*2* or MAC (Supplemental Tables 4 and 5).

Multiplicative and additive interaction effects between ADH1B alleles, MAC, and its impact on NAFLD severity

Figure 2 displays multiplicative effects between *ADH1B* alleles and alcohol intake (grams per day) and their impacts on risk of definite NASH (**Panel A**) and an NAS 4 (**Panel B**). We observe an inverse dose-dependent relationship between the amount of alcohol intake and risk of definite NASH or an NAS 4. Although alcohol consumption seems to reduce risk of NASH in patients with *ADH1B*1* or *ADH1B*2*, adjusted odds of having definite NASH or an NAS 4 for each gram of alcohol consumed are significantly lower in carriers of *ADH1B*2* (Adj. OR for definite NASH: 0.80 [95 CI: 0.68–0.94] and Adj. OR for an NAS 4: 0.83 [95% CI: 0.72–0.96]) than *ADH1B*1* (Adj. OR for definite NASH: 0.96 [95% CI: 0.93–0.99] and Adj. OR for an NAS 4: 0.96 [95% CI: 0.93–0.99]), P<.001 for difference in the effect of alcohol intake on definite NASH or an NAS 4 between alleles. Supplemental Tables 6 and 7 illustrate P values and confidence intervals for differences in predicted probabilities of having definite NASH and an NAS 4 according to both *ADH1B* alleles in each category of alcohol consumed.

We further explored multiplicative and additive interaction effects between MAC considered as a categorical variable (yes/no) and *ADH1B* alleles, and their impacts on NASH severity. MAC associates with lower risk for definite NASH or an NAS 4 in patients carrying *ADH1B*2* (Adj. ORs of 0.22 [95% CI: 0.10–0.49] or 0.28 [95% CI: 0.13–0.60], respectively) compared with *ADH1B*1* (Adj ORs of 0.71 [95% CI: 0.54–0.92] or 0.59 [95% CI: 0.34–1.05], respectively), P<.001 for difference in the effect of MAC on definite NASH

or an NAS 4 between alleles. ORs of 0.22 and 0.28 for the *ADH1B*2 × MAC multiplicative term mean that the combined effect of *ADH1B*2 and MAC is 0.22 and 0.28 times the product of the individual effects of *ADH1B*2 and MAC. Further analysis exploring interactions on an additive scale show a relative excess risk due to interaction (RERI) of −0.132 (95% CI: −0.589 to −0.089) and −0.153 (95% CI: −0.611 to −0.093) for the *ADH1B*2 × MAC interaction term and its risk on definite NASH or an NAS 4, respectively. These RERI mean that the risk of having definite NASH or an NAS 4 in *ADH1B*2 carriers who drink moderate alcohol is 0.132 or 0.153 less than if there were no interaction between MAC and *ADH1B*2. Supplemental Table 8 depicts results of *ADH1B*2-by-MAC (yes/no) interactions on a multiplicative or an additive scale.

To confirm the previous findings, we sought to explore the effect of *ADH1B*2 allele on the severity of NAFLD based on the history of MAC (yes/no), although the number of patients in each subgroup was too small for testing statistical significance on multivariable analysis. The direction and magnitude of the effect of *ADH1B*2 was similar to that seen in the whole cohort for hepatocyte ballooning degeneration, definite NASH or an NAS 4 among drinkers and non-drinkers. There was less clear relationship between *ADH1B*2 and fibrosis, probably because of the small number of patients with *ADH1B*2 in each group (Table 4).

The association between *ADH1B* alleles and liver histology severity in drinkers and non-drinkers remain significant and in general of similar magnitude in the multi-ethnic population than in non-Hispanic whites (supplemental Table 9).

Multiplicative and additive interactions effects between ADH1B alleles and BMI and its impact on NAFLD severity

The BMI average is significantly lower in moderate drinkers (34.18 ± 6.12) than non-drinkers (35.29 ± 6.55), P=.005. Moderate drinkers carrying *ADH1B*2 (32.93 ± 5.92) show lower mean BMI levels than those carrying *ADH1B*1 (34.29 ± 6.3), P=.047 (supplemental Figure 1). Because of the relationship between alcohol consumption, BMI and *ADH1B* alleles, we sought to explore the impact of a two-way interaction term of *ADH1B* alleles-by-BMI (kg/m^2) on predicted probabilities of definite NASH while controlling by alcohol consumption status, age and gender (Figure 3A). There is a positive association between BMI and risk of definite NASH, but interestingly the risk seems to be lower in *ADH1B*2 carriers who have a BMI of 37 (see bolded P values in the supplemental Table 10) compared with *ADH1B*1 carriers. We identify statistically significant interaction effects between *ADH1B*2 and BMI categorized as 37 and >37 kg/m^2 on probabilities of having definite NASH (P<.001) (Supplemental Table 11). An OR of 0.23 (95% CI: 0.12–0.43) for the *ADH1B*2 × BMI multiplicative term mean that the combined effect of *ADH1B*2 and BMI is 0.23 times the product of the individual effects of *ADH1B*2 and BMI. A RERI of −0.282 (95% CI: −1.00 to - 0.037) means that the risk of having definite NASH in *ADH1B*2 carriers who have BMI 37 is 0.282 less than if there were no interaction between BMI and *ADH1B*2.

To confirm the previous findings, we sought to explore the association between *ADH1B* alleles and selected NAFLD features among patients with or without BMI or >37. To do so, 4 groups ([1] BMI 37 with *ADH1B*1; [2] BMI 37 with *ADH1B*2; [3] BMI >37 with

ADH1B*1; [4] BMI >37 with *ADH1B*2*) were created (Figures 3B,3C and 3D). Among patients with BMI ≤ 37, the severity of NAFLD including categories of NASH (no steatohepatitis, and borderline and definite NASH) or NAFLD activity score (NAS <4 vs NAS ≥ 4) and fibrosis (from stage 0 to 4) is significantly lower amongst patients carrying *ADH1B*2* than those carrying *ADH1B*1*. Among individuals with BMI >37, no difference is observed between *ADH1B* alleles. Interestingly, the proportion of patients with history of MAC is not different among the 4 groups: BMI ≤ 37 (*ADH1B*2* [44%] vs *ADH1B*1* [40%], P=.466) and BMI >37 (*ADH1B*2* [28%] vs *ADH1B*1* [32%], P=.621), so alcohol consumption status does not seem to impact the association between *ADH1B* alleles and BMI on NAFLD severity.

DISCUSSION

The present study examines the relationship between a coding variant in alcohol dehydrogenase 1B (rs1229984), MAC, and risk for steatohepatitis and fibrosis severity among individuals with biopsy-proven NAFLD. Our data reveal that individuals with *ADH1B*2* have significantly decreased risk of several histological features of NAFLD including hepatocyte ballooning degeneration, lobular inflammation, steatohepatitis and global fibrosis, and this "protective" effect remains significant even after controlling by alcohol consumption status and other well-known confounding factors including age, gender, T2DM, BMI and *PNPLA3* rs738409 SNP. Our results also confirm that MAC reduces the severity of steatohepatitis in a dose-dependent manner in both *ADH1B* alleles, although greater benefits are observed in patients with the *ADH1B*2* allele.

A recent Mendelian Randomization study examined the effect of *ADH1B*2* allele on the amount of alcohol consumption as well as its association with liver histology severity among NAFLD patients. Authors reported that carriers of the *ADH1B*2* allele not only have lower consumption of alcohol but also decreased scores of histological steatosis, lobular inflammation and NAFLD activity score. They assumed that the effect of *ADH1B*2* on the liver histology is mediated exclusively by the amount of alcohol consumed and not by any other biological effect.[11] Surprisingly, we do not find a significant effect of *ADH1B*2* on different parameters related with alcohol consumption, and this finding could partly be explained by the low overall consumption observed in our cohort. To date, most of studies confirming protective effects of *ADH1B*2* on alcohol consumption are primarily focused on populations with heavier drinking patterns who are at higher risk of developing a tolerance and dependence to alcohol. Some studies exploring association between *ADH1B*2* and alcohol intake in populations with more heterogeneous patterns of drinking failed to detect protective effects on alcoholism, and hypothesized that it is likely due to low quantities of alcohol intake.[20,21]

Epidemiological studies have shown that light or moderate alcohol consumption, among male and female drinkers, are negatively associated with adiposity indicators (BMI, waist circumference and waist-to-hip ratio) compared to heavy drinking or abstention.[22] Whilst many factors such as gender, type, frequency and amount of alcohol consumed, physical activity, dietary habits, etc. may confound the alcohol-related effects on BMI, genetic aspects can also play a role in the predisposition of individuals to gain weight as result of

alcohol intake. Recent reports show that *ADH1B* polymorphisms affect susceptibility to alcoholism and may affect body weight via gene-associated differences in fuel utilization. [23,24] *ADH1B*2* allele is found to be a strong determinant of body weight in alcoholics.[24] The more rapid ethanol elimination associated with the *ADH1B*2* allele may result in less efficient utilization of ethanol as an energy source, which may result in lower weight gain compared with *ADH1B*1* carrier. Consistent with previous findings, our study shows a differential effect of *ADH1B*2* allele on body weight based on alcohol consumption status. Among patients with history of MAC, body weight is significantly lower in carriers of *ADH1B*2* compared with *ADH1B*1*, however no effect is seen among non-drinkers.

Our *ADH1B*-by-BMI interaction analysis also show a genetic independence between the two *ADH1B* alleles and its association with NAFLD severity. Although a positive dose-dependent association exists between body weight and NAFLD severity, the risk of having definite NASH seems to be attenuated among individuals with *ADH1B*2* allele as compared with *ADH1B*1*; however, this "protective" effect disappears in presence of BMI higher than 37 kg/m^2. Although the possible biological mechanisms underlying this finding are not entirely clear, it is increasingly accepted the relationship between increased levels of nondietary ethanol (derived from bacteria)[25] in obese individuals[26,27] and the severity of NAFLD.[27,28] Fasting plasma ethanol levels are strongly associated with body weight; and this association seems to be independent of dietary pattern or physical activity.[29,30]

In addition to increased obesity-related production of ethanol, other hypotheses suggest that modifications in insulin signaling followed by decreased ADH activity in the liver or adipose tissue could be responsible for an impaired ethanol metabolism.[31,32] In this regard, earlier studies conducted in rats suggest that activity of ADH is significantly reduced by high carbohydrate fat-free diet feeding compared with a normal chow diet. Furthermore in diabetic rats, ADH activity is found to be reduced by approximately 53% when compared with control animals.[31] The overproduction of endogenous ethanol along with impairments in insulin signaling in obese and diabetic patients may alter ADH activity in the liver, subsequently leading to an impaired ethanol metabolism and elevated blood ethanol levels in patients with NAFLD. The increased production of microbiota-related ethanol may result in upregulation of activity of the enzyme cytochrome P450 2E1, which catalyzes the oxidation of ethanol, but produce free radicals favoring oxidative damage, mitochondrial dysfunction and liver inflammation.[33] We hypothesized that individuals carrying the *ADH1B*2* allele have an ADH1B2 allozyme that exhibits a higher activity for ethanol oxidation, and therefore higher alcohol elimination rates than those with wild-type ADH1B alloenzyme. Thus, *ADH1B*2* carriers may, theoretically, be less vulnerable to the overproduction of endogenous or dietary alcohol, and therefore, exhibit less liver damage.[34] However, the mechanism for this protective effect is uncertain and the effect of *ADH1B*2* variant on the risk of NAFLD severity could be more complex. Because of the ADH1B2 allozyme exhibits a higher activity for ethanol oxidation, in presence of higher overproduction of endogenous alcohol in those with higher BMI or dietary alcohol, hepatic injury might result from high intrahepatic concentrations of acetaldehyde.[35] This finding might be a compatible explanation of the increased vulnerability to liver injury in carriers of *ADH1B*2* with higher BMI, although the relationship between BMI and *ADH1B* variants remains largely unknown and it warrants further investigation.

The *ADH1B* expression varies across multiple human tissues (http://BioGPS.org), including the liver and adipose tissue, among others. A recent study including human abdominal subcutaneous adipose tissue of 75 Mexican Americans found a strong and inverse relationship between *ADH1B* expression with obesity-related traits and insulin resistance. These direct links of *ADH1B* expression with various anthropometric measures, including waist circumference indicate a strong association of *ADH1B* with central obesity and insulin resistance, and a possible link with multiple metabolic traits, including T2DM and NAFLD. Unfortunately, the contribution of ADH1B activity in adipocytes to whole body ethanol clearance is not fully understood. However, it has been suggested that, in obese individuals, the adipose tissue may significantly be involve in alcohol elimination, although this could vary depending on genetic and/or environmental factors.[36] Therefore, down-regulation of *ADH1B* expression in the adipose tissue of obese individuals could potentially reduce the ethanol elimination and contribute to the worsening detrimental metabolic effects of increased adiposity.

Finally, it has been suggested that ADHs may also be involved in other non-alcohol related molecular pathways including all-trans-retinol and its derivatives, and lipid peroxidation products[37,38] which are known to be relevant to NAFLD pathophysiology. ADH are involved in the oxidation of retinol (ROL) to retinal (RAL), the first step in the biosynthesis of retinoic acid (RA).[39] RA is an active metabolite of vitamin A which has been implicated in the regulation of lipid metabolism and hepatic steatosis, inflammation and fibrosis in animal and humans studies.[40] Previous studies have suggested that ethanol is implicated in disruption of RA homeostasis; specifically it has the potential to inhibit ADH-mediated oxidation of retinol to retinal, the rate-limiting step in RA biosynthesis.[41,42] ROL is an important endogenous substrate for liver ADH, and its oxidation seems to be affected by genetic polymorphisms in *ADH1B*. In an elegant study, Chase, et al. showed that the ADH1B2 alloenzyme leads to greater ROL oxidation at different ethanol levels and, therefore, lesser impairment of the biogenesis of RA.[43]

We recognize several potential limitations of our study. First, the alcohol consumption was collected based on self-reported information, which may result in underestimates of the effects of individual SNPs due to alcohol consumption misclassification. Nonetheless, self-reported measures of alcohol intake have been shown to correlate strongly with the genetic risk for alcohol use disorders.[44] Second, a further limitation is our inability to examine the effect of *ADH1B* SNPs-by-alcohol intake associations on liver histology outcomes among Asians/Pacific Islanders/Hawaiians individuals. This was because the present study involves a relatively small number of Asians, Pacific Islanders and Hawaiians in whom the frequency of the wild-type *ADH1B* is lower. Thus, validations of our findings including more diversely ethnic populations are warranted. Unfortunately, the proportion of patients with *ADH1B*2* allele in each subgroup is smaller (n=55 in non-drinkers and n=35 in drinkers). We assumed that the case numbers are too limited to run analysis in these subpopulations while adjusting by relevant factors. The socioeconomic status has been recognized as a potential source of confounding, as high socioeconomic status is associated with MAC and better health outcomes. Finally, our analyses were not adjusted for educational attainment, smoking status, and various dietary and physical activity factors that may be reflective of socioeconomic status, so residual confounding is possible.

Despite these limitations, our study is based on a unique and very large cohort of non-Hispanic white individuals with biopsy confirmed NAFLD, who were all prospectively recruited in a similar manner, and assessed for their alcohol consumption using standardized questionnaires. We additionally confirmed previous associations between MAC and NAFLD liver histology severity in terms of effect size and direction, suggesting good internal validity.[3,4] Few studies have examined the association between the functional *ADH1B*2* variant and NAFLD histology severity in Caucasians because of its low prevalence, so our study provide interesting insights about the relationship between *ADH1B* alleles, MAC and NAFLD severity in this population.

In conclusion, our results support prior studies that suggest a protective effect of MAC on the risk of NASH and fibrosis. Further, *ADH1B*2* allele is associated with less severity of NAFLD and this protective effect seems to be independent of alcohol consumption; however, among moderate drinkers, the risk of NASH is different between each *ADH1B* alleles. Our results also support a positive dose-dependent relationship between BMI and risk of NASH and fibrosis, although this association seems to be significantly modified by *ADH1B*2*. Our study underscores the importance of gene-by-alcohol and/or -BMI associations and the risk of developing more severe histological phenotypes of NAFLD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations

NAFLD	Nonalcoholic fatty liver disease
MAC	Moderate alcohol consumption
ADH1B	Alcohol dehydrogenase class 1, beta polypeptide
HDL	High-density lipoprotein
HIV	Human immunodeficiency virus
PNPLA-3	Patatin-like phospholipase domain-containing 3
NASH	Nonalcoholic steatohepatitis
SNP	Single nucleotide polymorphism
BMI	Body mass index

T2DM	Type 2 diabetes mellitus
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
LDL	Low-density lipoprotein
Hb1Ac	Hemoglobin A1c
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
SD	Standard deviation
HWE	Hardy-Weinber equilibrium
OR	Odd ratio
CI	Confidence interval
ADH	Alcohol dehydrogenase
EtOH	Ethanol
ROL	Retinol
RAL	Retinal
RERI	Relative excess risk due to interaction
AP	Attibutable proportion to interaction

REFERENCES

1. Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 2016;64:73–84. [PubMed: 26707365]

2. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology 2018;67:328–357. [PubMed: 28714183]

3. Dunn W, Sanyal AJ, Brunt EM, et al. Modest alcohol consumption is associated with decreased prevalence of steatohepatitis in patients with non-alcoholic fatty liver disease (NAFLD). J Hepatol 2012;57:384–91. [PubMed: 22521357]

4. Mitchell T, Jeffrey GP, de Boer B, et al. Type and Pattern of Alcohol Consumption is Associated With Liver Fibrosis in Patients With Non-alcoholic Fatty Liver Disease. Am J Gastroenterol 2018;113:1484–1493. [PubMed: 29899440]

5. Sookoian S, Castano GO, Pirola CJ. Modest alcohol consumption decreases the risk of non-alcoholic fatty liver disease: a meta-analysis of 43 175 individuals. Gut 2014;63:530–2. [PubMed: 24026352]

6. Vu KN, Ballantyne CM, Hoogeveen RC, et al. Causal Role of Alcohol Consumption in an Improved Lipid Profile: The Atherosclerosis Risk in Communities (ARIC) Study. PLoS One 2016;11:e0148765. [PubMed: 26849558]

7. Greenfield JR, Samaras K, Hayward CS, et al. Beneficial postprandial effect of a small amount of alcohol on diabetes and cardiovascular risk factors: modification by insulin resistance. J Clin Endocrinol Metab 2005;90:661–72. [PubMed: 15522927]

8. Mukamal KJ, Jadhav PP, D'Agostino RB, et al. Alcohol consumption and hemostatic factors: analysis of the Framingham Offspring cohort. Circulation 2001;104:1367–73. [PubMed: 11560851]

9. Edenberg HJ. The genetics of alcohol metabolism: role of alcohol dehydrogenase and aldehyde dehydrogenase variants. Alcohol Res Health 2007;30:5–13. [PubMed: 17718394]

10. Li D, Zhao H, Gelernter J. Strong association of the alcohol dehydrogenase 1B gene (ADH1B) with alcohol dependence and alcohol-induced medical diseases. Biol Psychiatry 2011;70:504–12. [PubMed: 21497796]

11. Sookoian S, Flichman D, Castano GO, et al. Mendelian randomisation suggests no beneficial effect of moderate alcohol consumption on the severity of nonalcoholic fatty liver disease. Aliment Pharmacol Ther 2016;44:1224–1234. [PubMed: 27778410]

12. Sanyal AJ, Chalasani N, Kowdley KV, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med 2010;362:1675–85. [PubMed: 20427778]

13. Neuschwander-Tetri BA, Clark JM, Bass NM, et al. Clinical, laboratory and histological associations in adults with nonalcoholic fatty liver disease. Hepatology 2010;52:913–24. [PubMed: 20648476]

14. Berner MM, Kriston L, Bentele M, et al. The alcohol use disorders identification test for detecting at-risk drinking: a systematic review and meta-analysis. J Stud Alcohol Drugs 2007;68:461–73. [PubMed: 17446987]

15. Skinner HA, Allen BA. Alcohol dependence syndrome: measurement and validation. J Abnorm Psychol 1982;91:199–209. [PubMed: 7096790]

16. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–21. [PubMed: 15915461]

17. Brunt EM, Kleiner DE, Wilson LA, et al. Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. Hepatology 2011;53:810–20. [PubMed: 21319198]

18. Royston P marginscontplot: Plotting the marginal effects of continuous predictors. Stata Journal 2013;13:510–527.

19. Andersson T, Alfredsson L, Kallberg H, et al. Calculating measures of biological interaction. Eur J Epidemiol 2005;20:575–9. [PubMed: 16119429]

20. Jorgenson E, Thai KK, Hoffmann TJ, et al. Genetic contributors to variation in alcohol consumption vary by race/ethnicity in a large multi-ethnic genome-wide association study. Mol Psychiatry 2017;22:1359–1367. [PubMed: 28485404]

21. Hubacek JA, Pikhart H, Peasey A, et al. ADH1B polymorphism, alcohol consumption, and binge drinking in Slavic Caucasians: results from the Czech HAPIEE study. Alcohol Clin Exp Res 2012;36:900–5. [PubMed: 22150722]

22. Arif AA, Rohrer JE. Patterns of alcohol drinking and its association with obesity: data from the Third National Health and Nutrition Examination Survey, 1988–1994. BMC Public Health 2005;5:126. [PubMed: 16329757]

23. Duvigneaud N, Wijndaele K, Matton L, et al. Socio-economic and lifestyle factors associated with overweight in Flemish adult men and women. BMC Public Health 2007;7:23. [PubMed: 17324255]

24. Yokoyama A, Yokoyama T, Matsui T, et al. Alcohol dehydrogenase-1B genotype (rs1229984) is a strong determinant of the relationship between body weight and alcohol intake in Japanese alcoholic men. Alcohol Clin Exp Res 2013;37:1123–32. [PubMed: 23414439]

25. Cope K, Risby T, Diehl AM. Increased gastrointestinal ethanol production in obese mice: implications for fatty liver disease pathogenesis. Gastroenterology 2000;119:1340–7. [PubMed: 11054393]

26. Nair S, Cope K, Risby TH, et al. Obesity and female gender increase breath ethanol concentration: potential implications for the pathogenesis of nonalcoholic steatohepatitis. Am J Gastroenterol 2001;96:1200–4. [PubMed: 11316170]

27. Zhu L, Baker SS, Gill C, et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. Hepatology 2013;57:601–9. [PubMed: 23055155]

28. Yuan J, Chen C, Cui J, et al. Fatty Liver Disease Caused by High-Alcohol-Producing Klebsiella pneumoniae. Cell Metab 2019;30:675–688 e7. [PubMed: 31543403]

29. Engstler AJ, Aumiller T, Degen C, et al. Insulin resistance alters hepatic ethanol metabolism: studies in mice and children with non-alcoholic fatty liver disease. Gut 2016;65:1564–71. [PubMed: 26006114]

30. Troisi J, Belmonte F, Bisogno A, et al. Metabolomic Salivary Signature of Pediatric Obesity Related Liver Disease and Metabolic Syndrome. Nutrients 2019;11.

31. Lakshman MR, Chambers LL, Chirtel SJ, et al. Roles of hormonal and nutritional factors in the regulation of rat liver alcohol dehydrogenase activity and ethanol elimination rate in vivo. Alcohol Clin Exp Res 1988;12:407–11. [PubMed: 3044171]

32. Mezey E, Potter JJ, Mishra L, et al. Effect of insulin-like growth factor I on rat alcohol dehydrogenase in primary hepatocyte culture. Arch Biochem Biophys 1990;280:390–6. [PubMed: 2164356]

33. Baker SS, Baker RD, Liu W, et al. Role of alcohol metabolism in non-alcoholic steatohepatitis. PLoS One 2010;5:e9570. [PubMed: 20221393]

34. Lee SL, Chau GY, Yao CT, et al. Functional assessment of human alcohol dehydrogenase family in ethanol metabolism: significance of first-pass metabolism. Alcohol Clin Exp Res 2006;30:1132–42. [PubMed: 16792560]

35. Crabb DW, Matsumoto M, Chang D, et al. Overview of the role of alcohol dehydrogenase and aldehyde dehydrogenase and their variants in the genesis of alcohol-related pathology. Proc Nutr Soc 2004;63:49–63. [PubMed: 15099407]

36. Crabb DW, Zeng Y, Liangpunsakul S, et al. Ethanol impairs differentiation of human adipocyte stromal cells in culture. Alcohol Clin Exp Res 2011;35:1584–92. [PubMed: 21599713]

37. Gallego O, Belyaeva OV, Porte S, et al. Comparative functional analysis of human medium-chain dehydrogenases, short-chain dehydrogenases/reductases and aldo-keto reductases with retinoids. Biochem J 2006;399:101–9. [PubMed: 16787387]

38. Boleda MD, Saubi N, Farres J, et al. Physiological substrates for rat alcohol dehydrogenase classes: aldehydes of lipid peroxidation, omega-hydroxyfatty acids, and retinoids. Arch Biochem Biophys 1993;307:85–90. [PubMed: 8239669]

39. Yang ZN, Davis GJ, Hurley TD, et al. Catalytic efficiency of human alcohol dehydrogenases for retinol oxidation and retinal reduction. Alcohol Clin Exp Res 1994;18:587–91. [PubMed: 7943659]

40. Liu Y, Chen H, Wang J, et al. Association of serum retinoic acid with hepatic steatosis and liver injury in nonalcoholic fatty liver disease. Am J Clin Nutr 2015;102:130–7. [PubMed: 25948673]

41. Duester G Families of retinoid dehydrogenases regulating vitamin A function: production of visual pigment and retinoic acid. Eur J Biochem 2000;267:4315–24. [PubMed: 10880953]

42. Liu C, Russell RM, Seitz HK, et al. Ethanol enhances retinoic acid metabolism into polar metabolites in rat liver via induction of cytochrome P4502E1. Gastroenterology 2001;120:179–89. [PubMed: 11208727]

43. Chase JR, Poolman MG, Fell DA. Contribution of NADH increases to ethanol's inhibition of retinol oxidation by human ADH isoforms. Alcohol Clin Exp Res 2009;33:571–80. [PubMed: 19183134]

44. Kendler KS, Myers J, Dick D, et al. The relationship between genetic influences on alcohol dependence and on patterns of alcohol consumption. Alcohol Clin Exp Res 2010;34:1058–65. [PubMed: 20374207]

What you need to know:

Background and Context:

The *ADH1B*2* allele of the alcohol dehydrogenase 1B gene is associated with higher alcohol metabolism and might affect the relationship between moderate alcohol consumption and severity of NAFLD.

New Findings:

In an analysis of data from the nonalcoholic steatohepatitis (NASH) clinical research network, from 1153 patients with NAFLD, the authors found that *ADH1B*2* reduces the risk of NASH and fibrosis in adults with NAFLD regardless of alcohol consumption status.

Limitations:

This was an analysis of mostly white patients; larger studies of more diverse groups are needed. Studies are also needed to determine them mechanisms by which the *ADH1B*2* allele might reduce risk of severe NAFLD.

Impact:

These observations might shed further light on the pathogenesis of NASH and facilitate the development of new therapies.
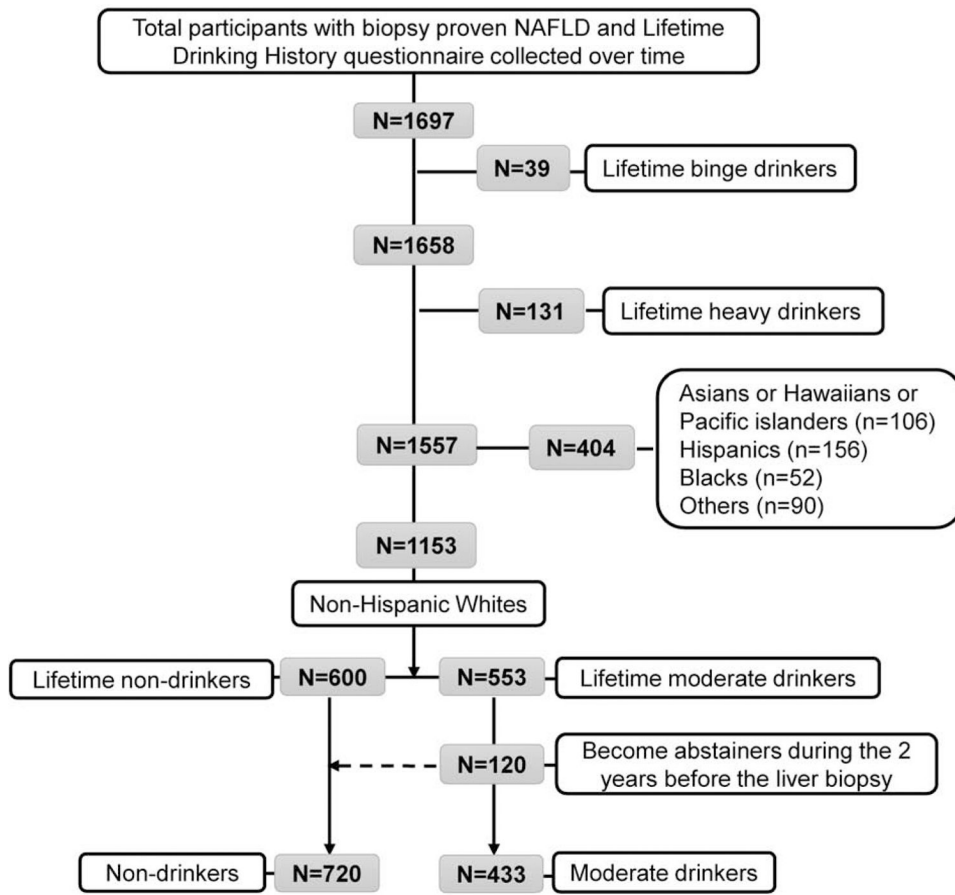
Figure 1.
Flow chart of patient selection.
Abbreviations: NAFLD, non-alcoholic fatty liver disease.
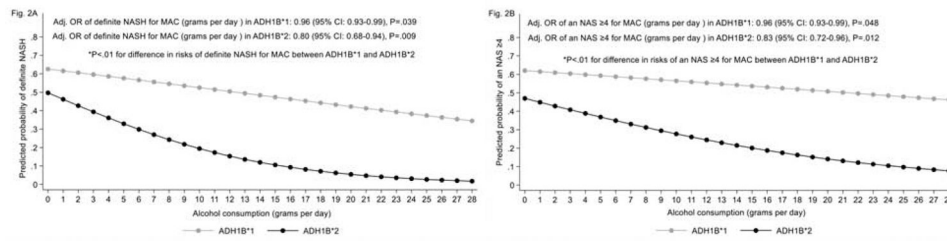
Figure 2.

Multiplicative effects between *ADH1B* alleles and MAC, and its impact on liver histology severity.

(**A**) Predicted probability of definite steatohepatitis based on alcohol consumption (g/day) and *ADH1B* alleles.

(**B**) Predicted probability of an NAS ≥ 4 based on alcohol consumption (g/day) and *ADH1B* alleles.

Abbreviations: ADH1B, alcohol dehydrogenase class I, beta polypeptide; **NAS**, NAFLD activity score; **OR**, odd ratio; **MAC**, moderate alcohol consumption.

Logistic regression models were used to compute predicted probabilities of histological outcomes and margins command to create a visual display of results. Circles on the lines represent average adjusted predictions of definite steatohepatitis or an NAS ≥ 4 for each gram of alcohol consumed according to *ADH1B* alleles. All analyses are adjusted by gender, age and body mass index (kg/m^2).

* P corresponds with difference of the effect of alcohol consumption (g/day) on definite steatohepatitis or an NAS ≥ 4 between *ADH1B* alleles.
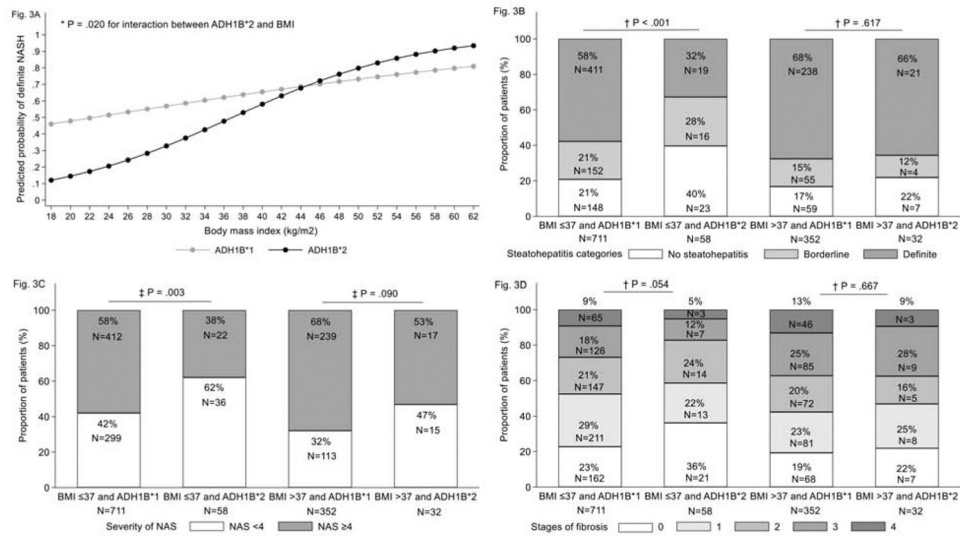
Figure 3.
Association or interaction effects between *ADH1B* alleles and BMI (kg/m^2), and its impact on selected histological features of NAFLD.

(**A**) Predicted probability of definite NASH based on BMI (kg/m^2) and *ADH1B* alleles.

(**B**) Association between BMI (kg/m^2), *ADH1B* alleles and categories of steatohepatitis.

(**C**) Association between BMI (kg/m^2), *ADH1B* alleles and an NAS 4.

(**D**) Association between BMI (kg/m^2), *ADH1B* alleles and fibrosis stages.

Abbreviations: ADH1B, alcohol dehydrogenase class I, beta polypeptide, **BMI**, body mass index; **NAS**, NAFLD activity score.

Logistic regression models were used to compute predicted probabilities of definite NASH and margins command to create a visual display of results. Circles on the lines represent average adjusted predictions of definite NASH for every 2 units of change in BMI (kg/m^2) according to *ADH1B* alleles. All analyses are adjusted by gender, age and alcohol consumption.

* P value corresponds with adjusted logistic regression model.

† P value corresponds with Cochran-Armitage test for trend.

‡ P value corresponds with Chi-square statistic test.

Table 1.

Baseline characteristics according to *ADH1B*1* or *ADH1B*2* alleles among non-Hispanic Whites.

Variables	*ADH1B* alleles		
	*ADH1B*2* (AA/AG)	*ADH1B*1* (GG)	P value[*]
	N=90	N=1063	
PNPLA3 rs738409 genotypes, n (%)			.829
CC	32 (35.6)	345 (32.5)	
GC	39 (43.3)	489 (46)	
GG	19 (21.1)	229 (21.5)	
Age, years	51.12 ± 12.31	50.70 ± 11.39	.735
Gender, n (%)			.191
Male	39 (43)	387 (36)	
Female	51 (57)	676 (64)	
Body mass index (kg/m^2)	34.63 ± 6.53	34.89 ± 6.41	.710
Waist (cm)	110.36 ± 13.77	110.38 ± 14.27	.989
Type 2 diabetes mellitus, n (%)	29 (32)	379 (36)	.513
Hypertension, n (%)	46 (51)	639 (60)	.095
Lab panel			
Alanine aminotransferase (U/L)	69.03 ± 48.72	70.07 ± 47.12	.841
Aspartate aminotransferase (U/L)	51.02 ± 33.68	51.38 ± 32.21	.920
Triglycerides (mg/dl)[†]	205.28 ± 150.12	184.01 ± 180.25	.068
Total cholesterol (mg/dl)[†]	195.44 ± 45.28	192.13 ± 44.23	.573
HDL cholesterol (mg/dl)[†]	43.09 ± 10.25	43.36 ± 11.71	.989
LDL cholesterol (mg/dl)[†]	116.55 ± 38.33	115.67 ± 37.22	.959
Fasting glucose (mg/dl)[†]	103.99 ± 32.27	108.20 ± 37.30	.242
Insulin (mlU/L)[†]	19.72 ± 13.67	24.91 ± 25.33	.040
HOMA-IR[†]	5.42 ± 5.84	7.21 ± 9.98	.033
HbAlc (%)[†]	6.05 ± 1.07	6.18 ± 1.14	.256
Most recent history of alcohol consumption (2 years preceding the liver biopsy)			
Moderate alcohol consumers, n (%)	35 (39)	398 (37)	.785
Drinks per day (average)	0.78 ± 1.28	0.72 ± 1.22	.641
Days per month (average)	1.64 ± 3.45	1.55 ± 3.37	.802
Quantity-frequency index (average)	3.36 ± 7.26	2.99 ± 6.98	.630
Maximum number of drinks (average)	1.46 ± 2.74	1.27 ± 2.34	.470
Gram per day (average)	1.68 ± 3.63	1.49 ± 3.49	.630
Lifetime history of alcohol consumption			
Moderate alcohol consumers, n (%)	46 (51)	507 (48)	.533
Drinks per day (average)	1.26 ± 1.63	1.19 ± 1.60	.667
Days per month (average)	2.59 ± 3.53	2.58 ± 3.97	.979
Quantity-frequency index (average)	6.45 ± 9.63	6.24 ± 10.21	.847

Variables	ADH1B alleles		
	ADH1B*2 (AA/AG)	ADH1B*1 (GG)	P value*
	N=90	N=1063	
Maximum number of drinks (average)	2.40 ± 3.30	2.21 ± 3.07	.587
Gram per day (average)	3.22 ± 4.81	3.12 ± 5.10	.847

Abbreviations: ADH1B,alcohol dehydrogenase class 1 beta subunit; **HDL**, high-density lipoprotein; **LDL**, low-density lipoprotein; **PNPLA3**, patatin-like phospholipase domain-containing protein 3.

Quantity-frequency index: alcohol consumption frequency (number of days drank per month) × quantity (number of drinks usually drank on each occasion). Because of the skewed nature of the data, the quantity, frequency, and QFI scores were log-transformed [ln (score + 1)] for comparative analysis.

*
T test or Wilcoxon rank-sum test used for continuous variables. Chi-square statistic test used forcategorical variables. Mantel-Haenszel trend test used for binary categorical variables with three or more groups.

†
Log-transformed variables.

Table 2.

Influence of alcohol consumption or *ADH1B* alleles on liver histology severity among non-Hispanic Whites. Results based on univariate and multivariable analysis.

Histopathological report	Most recent history of alcohol consumption					ADH1B alleles				
	Moderate drinkers N=433	Non-drinkers N=720	P value*	Adj. OR (95% CI)†	P value	ADH1B*2 (AA-AG) N=90	ADH1B*1 (GG) N=1063	P value*	Adj. OR (95% CI)†	P value
Steatosis, n (%)			.074	1.24 (0.99–1.56)	.062			.568	0.93 (0.63–1.38)	.724
<5%	6 (1.4)	15 (2.1)				1 (1.1)	20 (1.9)			
5–33%	142 (32.8)	278 (38.6)				34 (37.8)	386 (36.3)			
33–66%	168 (38.8)	245 (34)				36 (40)	377 (35.5)			
>66%	117 (27)	182 (25.3)				19 (21.1)	280 (26.3)			
Lobular inflammation, n (%)			.317	0.98 (0.77–1.25)	.898			.033	0.63 (0.41–0.98)	.044
No foci	2 (0.5)	0 (0)				1 (1.1)	1 (0.1)			
<2 foci/200x	233 (53.8)	373 (51.8)				56 (62.2)	550 (51.7)			
2–4 foci/200x	154 (35.6)	265 (36.8)				26 (28.9)	393 (37)			
>4 foci/200x	44 (10.2)	82 (11.4)				7 (7.8)	119 (11.2)			
Ballooning, n (%)			<.001	0.82 (0.65–1.03)	.095			.002	0.53 (0.35–0.80)	.003
None	168 (38.8)	215 (29.8)				45 (50)	338 (31.8)			
Few	123 (28.4)	197 (27.4)				19 (21.1)	301 (28.3)			
Many	142 (32.8)	308 (42.8)				26 (28.9)	424 (39.9)			
Portal inflammation, n (%)			.014	0.85 (0.66–1.10)	.227			.935	1.04 (0.67–1.62)	.850
None	56 (12.9)	70 (9.7)				8 (8.9)	118 (11.1)			
Mild	284 (65.6)	456 (63.3)				61 (67.8)	679 (63.9)			
>Mild	93 (21.5)	194 (27)				21 (23.3)	266 (25)			
Fibrosis stages, n (%)			<.001	0.68 (0.55–0.85)	.001			.085	0.69 (0.47–0.99)	.050
0	120 (27.7)	138 (19.2)				28 (31.1)	230 (21.6)			
1	133 (30.7)	180 (25)				21 (23.3)	292 (27.5)			
2	74 (17.1)	164 (22.8)				19 (21.1)	219 (20.6)			
3	73 (16.9)	154 (21.4)				16 (17.8)	211 (19.8)			

Histopathological report	Most recent history of alcohol consumption					ADH1B alleles				
	Moderate drinkers N=433	Non-drinkers N=720	P value*	Adj. OR (95% CI)†	P value	ADH1B*2 (AA-AG) N=90	ADH1B*1 (GG) N=1063	P value*	Adj. OR (95% CI)†	P value
4	33 (7.6)	84 (11.6)				6 (6.7)	111 (10.4)			
Mallory-Denk bodies, n (%)			.001	0.82 (0.62–1.08)	.155			.061	0.63 (0.37–1.05)	.081
Rare	314 (73)	454 (63)				68 (76)	700 (66)			
Many	119 (27)	266 (37)				22 (24)	363 (34)			
Steatohepatitis, n (%)			<.001	0.73 (0.57–0.94)	.013			.001	0.52 (0.34–0.78)	.002
No steatohepatitis	105 (24.2)	132 (18.3)				30 (33.3)	207 (19.5)			
Borderline steatohepatitis	102 (23.6)	125 (17.4)				20 (22.2)	207 (19.5)			
Definite steatohepatitis	226 (52.2)	463 (64.3)				40 (44.4)	649 (61.1)			
Definite steatohepatitis, n (%)	226 (52.2)	463 (64.3)	<.001	0.70 (0.54–0.91)	.008	40 (44.4)	649 (61.1)	.001	0.50 (0.31–0.79)	.003
NAS ≥4, n (%)	236 (54)	454 (63)	.004	0.77 (0.60–0.99)	.048	39 (43)	651 (61)	.001	0.48 (0.30–0.75)	.001
Advanced fibrosis (F ≥3), n (%)	106 (25)	238 (33)	.002	0.77 (0.58–1.02)	.073	22 (24)	322 (30)	.244	0.74 (0.44–1.26)	.274

Abbreviations: ADH1B, alcohol dehydrogenase class 1 beta subunit; **OR**, odd ratio; **CI**, confidence interval; **NAS**, NAFLD activity score.

*
Chi-square statistic test used for binary and Cochran-Armitage trend test used for ordered alternatives.

†
Covariate-adjusted ordered or binary logistic regression. Adjustment by age, gender, BMI (kg/m²), type 2 diabetes mellitus and *PNPLA3* rs738409. Both the most recent history of alcohol consumption and *ADH1B* alleles were included in the same multivariable adjusted log models.

Table 3.

Association between different cutoffs of alcohol consumption and baseline characteristics among non-Hispanic Whites.

Variable	Most recent history of alcohol consumption (g/day)				P value[*]
	0 g/day N=720	0–6.9 g/day N=351	7–13.9 g/day N=60	14–28 g/day N=22	
ADH1B, n (%)					.512
ADH1B*1	665 (92)	325 (93)	51 (85)	22 (96)	
ADH1B*2	55 (8)	25 (7)	9 (15)	1 (4)	
*PNPLA3 rs*738409 genotypes, n (%)					.842
GG	146 (20.3)	87 (24.8)	10 (16.7)	5 (22.7)	
CG	340 (47.2)	147 (41.9)	33 (55)	8 (36.4)	
CC	234 (32.5)	117 (33.3)	17 (28.3)	9 (40.9)	
Body mass index (kg/m^2)	35.29 ± 6.55	34.44 ± 6.27	32.64 ± 5.03	34.28 ± 6.20	.007
Type 2 diabetes mellitus, n (%)	284 (39)	105 (30)	16 (27)	3 (13)	<.001
Hypertension, n (%)	455 (63)	184 (53)	30 (50)	15 (68)	.003
Lab panel					
Alanine aminotransferase (U/L)	68.06 ± 45.88	72.83 ± 50.80	71.93 ± 42.74	82.45 ± 41.48	.251
Aapartate aminotransferase (U/L)	52.18 ± 34.11	50.05 ± 29.51	49.93 ± 29.93	48.95 ± 17.93	.735
Triglycerides (mg/dl)[†]	187.22 ± 175.51	185.73 ± 189.36	152.10 ± 77.90	224.77 ± 258.14	.192
Total cholesterol (mg/dl)[†]	193.64 ± 46.00	188.80 ± 40.89	195.38 ± 44.94	200.05 ± 36.22	.366
HDL cholesterol (mg/dl)[†]	43.24 ± 11.43	43.03 ± 11.62	45.78 ± 11.93	44.86 ± 15.06	.359
LDL cholesterol (mg/dl)[†]	116.68 ± 38.11	112.65 ± 35.46	120.32 ± 38.66	122.05 ± 33.34	.385
Fasting glucose (mg/dl)[†]	108.33 ± 38.54	107.65 ± 34.68	106.07 ± 34.99	101.32 ± 21.34	.872
HOMA-IR[†]	7.19 ± 9.48	7.19 ± 10.37	4.82 ± 4.07	4.64 ± 3.58	.016
HbA1c (%)[†]	6.22 ± 1.17	6.14 ± 1.09	5.93 ± 0.97	5.98 ± 1.13	.189
Histopathological reports					
Steatosis, n (%)					.112
<5%	15 (2.1)	5 (1.4)	1 (1.7)	0 (0)	
5–33%	278 (38.6)	114 (32.5)	21 (35)	7 (31.8)	
33–66%	245 (34)	138 (39.3)	20 (33.3)	10 (45.5)	

Variable	Most recent history of alcohol consumption (g/day)				P value*
	0 g/day	0–6.9 g/day	7–13.9 g/day	14–28 g/day	
	N=720	N=351	N=60	N=22	
>66%	182 (25.3)	94 (26.8)	18 (30)	5 (22.7)	.433
Lobular inflammation, n (%)					<.001
No foci	0 (0)	1 (0.3)	1 (1.7)	0 (0)	
<2 foci/200x	373 (51.8)	189 (53.8)	35 (58.3)	9 (41)	
2–4 foci/200x	265 (36.8)	124 (35.4)	18 (30)	12 (54.5)	
>4 foci/200x	82 (11.4)	37 (10.5)	6 (10)	1 (4.5)	
Ballooning, n (%)					<.001
None	215 (29.9)	130 (37)	29 (48.3)	9 (40.9)	
Few	197 (27.4)	98 (27.9)	15 (25)	10 (45.5)	
Many	308 (42.7)	123 (35.1)	16 (26.7)	3 (13.6)	
Portal inflammation, n (%)					<.001
None	70 (9.8)	39 (11.1)	11 (18.3)	6 (27.3)	
Mild	456 (63.3)	229 (65.2)	42 (70)	13 (59.1)	
>Mild	194 (26.9)	83 (23.6)	7 (11.7)	3 (13.6)	
Fibrosis stages, n (%)					<.001
0	138 (19.1)	93 (26.5)	23 (38.3)	4 (18.2)	
1	180 (25)	106 (30.2)	19 (31.7)	8 (36.4)	
2	164 (22.8)	59 (16.8)	11 (18.3)	4 (18.2)	
3	154 (21.4)	63 (17.9)	4 (6.7)	6 (27.3)	
4	84 (11.7)	30 (8.6)	3 (5)	0 (0)	
Mallory-Denk bodies, n (%)					<.001
Rare	454 (63)	249 (71)	45 (75)	20 (91)	
Many	266 (37)	102 (29)	15 (25)	2 (9)	
Category of steatohepatitis, n (%)					<.001
No steatohepatitis	132 (18.3)	80 (22.8)	22 (36.7)	3 (13.6)	
Borderline steatohepatitis	125 (17.4)	82 (23.4)	12 (20)	8 (36.4)	
Definite steatohepatitis	463 (64.3)	189 (53.8)	26 (43.3)	11 (50)	
Definite steatohepatitis	463 (64)	189 (54)	26 (43)	11 (50)	<.001
NAS 4	454 (63)	197 (56)	27 (45)	12 (54)	.003

Variable	Most recent history of alcohol consumption (g/day)				
	0 g/day	0–6.9 g/day	7–13.9 g/day	14–28 g/day	P value[*]
	N=720	N=351	N=60	N=22	
Advanced fibrosis (F 3), n (%)	238 (33)	93 (27)	7 (12)	6 (27)	.001

Abbreviations: ADH1B, alcohol dehydrogenase class 1 beta subunit; **HDL**, high-density lipoprotein; **LDL**, low-density lipoprotein;

PNPLA3, patatin-like phospholipase domain-containing protein 3; **NAS**, NAFLD activity score.

[*] Cochran-Armitage trend test used for ordered alternatives, Mantel-Haenszel trend test used for binary categorical variables with 3 or more groups, and one-way ANOVA used for continuous variables with Bonferroni correction for multiple comparisons.

[†] Log-transformed variables.

Table 4.

Association between *ADH1B* alleles and liver histology phenotypes among non-Hispanic whites. Sensitivity analysis based on the most recent history of alcohol consumption.

Histopathological reports	Non-drinkers			Moderate drinkers		
	*ADH1B*2* (AA-AG) N=55	*ADH1B*1* (GG) N=665	P value[*]	*ADH1B*2* (AA-AG) N=35	*ADH1B*1* (GG) N=398	P value[*]
Steatosis, n (%)			.950			.380
<5%	1 (1.8)	14 (2.1)		0 (0)	6 (1.5)	
5–33%	21 (38.2)	257 (38.6)		13 (37.1)	129 (32.4)	
33–66%	20 (36.4)	225 (33.8)		16 (45.8)	152 (38.2)	
>66%	13 (23.6)	169 (25.4)		6 (17.1)	111 (27.9)	
Lobular inflammation, n (%)			.016			.716
No foci	0 (0)	0 (0)		1 (2.9)	1 (0.3)	
<2 foci/200x	36 (65.5)	337 (50.7)		20 (57.1)	213 (53.5)	
2–4 foci/200x	17 (30.9)	248 (37.3)		9 (25.7)	145 (36.4)	
>4 foci/200x	2 (3.6)	80 (12)		5 (14.3)	39 (9.8)	
Ballooning, n (%)			.177			.001
None	22 (40)	193 (29)		23 (65.7)	145 (36.4)	
Few	12 (21.8)	185 (27.8)		7 (20)	116 (29.1)	
Many	21 (38.2)	287 (43.2)		5 (14.3)	137 (34.4)	
Portal inflammation, n (%)			.722			.542
None	4 (7.3)	66 (9.9)		4 (11.4)	52 (13.1)	
Mild	39 (70.9)	417 (62.7)		22 (62.9)	262 (65.8)	
>Mild	12 (21.8)	182 (27.4)		9 (25.7)	84 (21.1)	
Fibrosis stages, n (%)			.288			.159
0	13 (23.6)	125 (18.8)		15 (42.9)	105 (26.4)	
1	12 (21.8)	168 (25.3)		9 (25.7)	124 (31.2)	
2	16 (29.1)	148 (22.3)		3 (8.6)	71 (17.8)	
3	10 (18.2)	144 (21.7)		6 (17.1)	67 (16.8)	
4	4 (7.3)	80 (12)		2 (5.7)	31 (7.8)	
Mallory-Denk bodies, n (%)			.500			.027
Rare	37 (67)	417 (63)		31 (89)	283 (71)	
Many	18 (33)	248 (37)		4 (11)	115 (29)	
Steatohepatitis, n (%)			.138			<.001
No steatohepatitis	14 (25.5)	118 (17.7)		16 (45.7)	89 (22.4)	
Borderline steatohepatitis	10 (18.2)	115 (17.3)		10 (28.6)	92 (23.1)	
Definite steatohepatitis	31 (56.4)	432 (65)		9 (25.7)	217 (54.5)	
Definite steatohepatitis, n (%)	31 (56)	432 (65)	.201	9 (26)	217 (55)	.001
NAS 4, n (%)	29 (53)	425 (64)	.099	10 (29)	226 (57)	.001
Advanced fibrosis (F 3), n (%)	14 (25)	224 (34)	.212	8 (23)	98 (25)	.816

Abbreviations: ADH1B, alcohol dehydrogenase class 1 beta subunit; **NAS**, NAFLD activity score

[*] Chi-square statistic test used for binary and Cochran-Armitage trend test used for ordered alternatives.